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OBSERVATIONS ON THE STUDY OF VARIETAL DIFFERENCES IN THE MALTING QUALITY OF BARLEY. PART IV[1]

BY HENRY R. SALLANS[2] AND J. ANSEL ANDERSON[2]

Abstract

An investigation was undertaken to compare the relative magnitudes of the differential effects of environment (*i.e.*, stations) and of malting methods on the malting quality of barley varieties. Samples of Olli, O.A.C. 21, Hannchen, and Regal from four stations were malted by eight methods representing the combinations of germinating at 50° and 56° F., at 42 and 46% moisture, and for six and nine days.

Statistical analyses showed that, with respect to diastatic activity, wort nitrogen, and extract yield, the variations due to the differential effect of environment on varieties were greater than the variations due to the differential effect of malting methods on varieties. As a result of this study and earlier ones, it appears that the former effect is the limiting factor in studies of the comparative malting qualities of varieties. The latter effect is of less importance but must be kept in mind if errors in the interpretation of the results of routine tests are to be avoided. In general, it appears that if routine tests show that the mean values for any variety, when grown at 12 stations representing a reasonable range of environment, differ from the values for the standard variety by more than 1% in extract, or 10% in diastatic activity or wort nitrogen, a real difference exists between the varieties, which cannot be overcome by any reasonable change in malting conditions.

As a result of previous studies in this series (2, 3, 5), it has been suggested that the differential effect of malting methods on varieties may be an appreciable source of error in the interpretation of the results of routine tests in which all samples are malted by one method. It seems probable, however, that the limiting factor in studies of the comparative malting qualities of varieties is the differential effect of environment on them.

Theoretically it should be possible to make an adequate test of this hypothesis by means of one large investigation involving malting, under a wide range of conditions, samples of a large number of varieties grown at a number of points representing a wide range of environments. In practice, the difficulty of obtaining a suitable series of sufficiently large samples of barley, the limited capacity of available malting equipment, and the amount of time and number of analysts required, make it almost impossible to carry out one single investigation of adequate size. It has accordingly been necessary to resort to the use of several small investigations. Considered individually

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these are not particularly convincing, but considered together, they provide a good deal of support for the hypothesis.

This paper presents the results of a fourth study in which samples of four barley varieties from four experimental stations were malted by eight methods. An attempt was made to determine whether circumstances might exist in which the differential effect of malting methods on varieties, rather than the differential effect of environment on varieties, would be the limiting factor in investigations of varietal differences in barley. For this reason an attempt was made to exaggerate the former effect. The malting treatments were selected to cover a very wide range of conditions: from a 6-day period of slow growth at 50° F., 42% moisture and with minimum aeration, to a 9-day period of rapid growth at 56° F., 46% moisture and with liberal aeration. All the malts grown under the first set of conditions were definitely undermodified, whereas all those grown under the last set of conditions were overmodified. The range of conditions was thus wider than that which would be selected for making commercial malts from these barleys, and the differential effect of malting methods on varieties was thus exaggerated.

Moreover, further exaggeration was obtained by using previous information on some 20 varieties grown in Canada to select four that promised to differ very widely in their reactions to changes in malting conditions, including amongst these one two-rowed variety, Hannchen. In our opinion most random selections of four varieties would tend to show less differential response than the four varieties used in the present study. This would apply particularly to sets composed entirely of six-rowed or entirely of two-rowed varieties. In general, except for certain experimental purposes, it seems unwise to attempt to compare the malting qualities of two- and six-rowed varieties by malting them by the same method, since, if this is satisfactory for one class, it will hardly do justice to the other. The results showed that the two-rowed variety, Hannchen, contributed more than its share to the interaction between malting treatments and varieties, particularly with respect to malting loss and wort nitrogen. Thus if Hannchen had been replaced by a six-rowed variety, it seems probable that the differential effect of treatments on varieties would have been reduced.

On the other hand, no attempt was made to exaggerate the differential effect of environment on varieties. So far as can be determined, selection of four varieties which differed widely in their reactions to changes in malting conditions, did not also result in selection of varieties which differed abnormally in their reactions to changes in environment. In addition, it appears that the four stations selected represent a fair sample of the range of environments over which varieties are commonly compared in Canada, and the differential effect of stations on varieties is of the order usually found in investigations of this type.

It is thus apparent that in the present investigation a definite attempt was made to disprove the hypothesis in which we are interested, namely, that the differential effect of environment on varieties, rather than the differential

effect of malting treatment on varieties, is the limiting factor in studies of the comparative malting qualities of different barley varieties. In spite of this attempt the results served to substantiate the hypothesis with respect to diastatic activity, wort nitrogen and extract.

The investigation was so designed that certain interrelations between the effects of germination time, temperature, and moisture could be investigated by statistical methods. Although the results thus obtained have little bearing on the hypothesis under investigation, they appeared to be worth reporting briefly.

Materials

The following four varieties were selected for study: O.A.C. 21, the six-rowed, rough-awned Manchurian type variety which is accepted as the standard of malting quality in Canada; Olli, a similar variety to O.A.C. 21 but of earlier maturing habit and with rather smaller kernels, which seems promising from the malting viewpoint; Regal, a six-rowed, smooth-awned variety which Canadian maltsters consider unsatisfactory; and Hannchen, a two-rowed, rough-awned variety of which considerable quantities are malted in the United States.

Samples of these four varieties, grown in adjacent plots, were obtained from four experimental stations, namely: Nappan, Nova Scotia; Kapuskasing, Ontario; Ottawa, Ontario; and Lacombe, Alberta. The thin kernels were removed from the samples by passing them over a ring grader with rings 5/64 in. apart. A Boerner sampler was used in preparing sub-samples.

Equipment and Methods

Malting Equipment

The malts were made in laboratory equipment at the National Research Laboratories, Ottawa. One steep-tank for 16 samples, two germinators, each for 8 samples, and one kiln for 8 samples, were available for the investigation. The germinators and kiln have already been described (1).

New steeping equipment was built just before the present investigation was started and will be described in detail in a future paper. It provides for automatic aeration of samples and automatic changing of steepwater. In addition it permits the steeping of individual samples to be started automatically at predetermined times (within ± 5 min.). This feature, in conjunction with pilot experiments designed to determine the length of time required to bring a sample to the required moisture content, makes it readily possible to steep all samples to within $\pm 0.5\%$ of the required moisture content at the same time. The exact moisture content required can then be obtained by adding a few grams of water by sprinkling with an atomizer or by removing a few grams with blotting paper. For the present investigation, samples representing 250 gm. of barley dry matter were steeped to 42 or 46% moisture at 50° F., with a change of water and 1 hr. of aeration every 12 hr.

Malting Treatments

The eight malting treatments used consisted of the combinations of:—two germination times, 6 and 9 days; two germination temperatures, 50° and 56° F.; and two moisture levels, 42 and 46%.

In order to accentuate differences in growth rate, the samples that were steeped to 42% moisture were given less aeration and no watering. They were germinated in cylindrical galvanized iron containers, 6 in. long by 6 in. in diameter, containing eight $\frac{1}{8}$-in. holes. Aeration and evaporation were thus reduced to a minimum and the samples grew relatively slowly but continuously and without requiring water. The samples that were steeped to 46% moisture were germinated in 200-hole containers. Considerable aeration was thus provided, and as evaporation took place all malts were sprinkled with 20 gm. of water after 72 hr., and the 9-day malts were sprinkled a second time with 10 gm. of water after 144 hr.

All malts were dried in the same kiln in rotating wire-mesh cages under the following time-temperature schedule: 0 to 6 hr., temperature rising at constant rate from 90° to 125° F.; 6 to 12 hr., at 125° F.; 12 to 16 hr., temperature rising at constant rate to 140° F.; 16 to 21 hr., at 140° F.; 21 to 22 hr., temperature rising at constant rate to 170° F.; 22 to 26 hr., at 170° F.

Malting Plan

It seemed best to make in each batch the 32 malts representing eight treatments applied to four varieties from one station. This could be done as follows: by germinating four samples steeped to 42% moisture and four samples steeped to 46% moisture in one chamber at 50° F.; by germinating a corresponding set of eight samples in the second chamber at 56° F.; and by removing and kilning half of each sample after six days and the remaining halves after nine days. However, this procedure could not be followed, since it involved kilning 16 half-samples simultaneously whereas the kiln accommodated only eight half-samples. It was accordingly necessary to malt the half-batch grown at 56° F. five days later than the half-batch grown at 50° F. This schedule made it possible to remove the green malts in lots of eight and thus to dry all malts in one kiln.

Four batches, one for each station, were required to make one set of 128 malts, and four more batches were required to make the duplicate set. The first and second sets of four batches were malted in random order, and the samples were also arranged in random order within half-batches.

Analytical Methods

The moisture content and extract yield of the malts were determined by the Official Methods of the American Society of Brewing Chemists. Diastatic activity was determined by a ferricyanide modification of the official method (4). Wort nitrogen was determined by a Kjeldahl determination made on 25 ml. of wort after acidification and evaporation to a thin syrup, and is

reported as percentage of malt dry matter. Malting loss was calculated from data on the dry weights and moisture contents of the barley samples and the finished malts.

Single determinations of diastatic activity and malting loss were made on each of the duplicate malts. The precision of the malting methods was thus established by use of the two properties most sensitive to variations in malting conditions. Owing to press of other work the remaining portions of duplicate malts were mixed and single determinations of extract and wort nitrogen were made.

Results

Diastatic Activity

The results for diastatic activity are presented in Fig. 1 by means of a family of 25 histograms. These are arranged in tabular form, the columns showing the results for different malting treatments and the means over all treatments, and the rows showing the results for samples from different stations and the means over all stations.

Each individual histogram contains four columns which represent the four varieties: from left to right, Olli, O.A.C. 21, Hannchen, and Regal. The

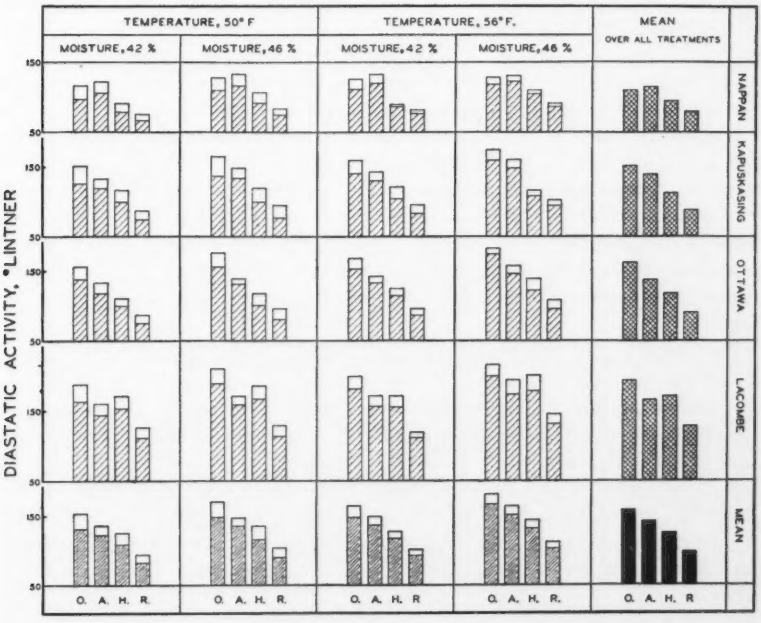

FIG. 1. *Histograms for diastatic activity illustrating the differential effects of stations and malting treatments on varieties. The four columns in each histogram represent four varieties:* O = *Olli*, A = *O.A.C. 21*, H = *Hannchen, and* R = *Regal. Tops of hatched portions represent six-day malts and tops of blank portions represent nine-day malts.*

hatched portion of each column represents the results for six-day malts and the white portion represents the additional effect of growing the malts for three more days. It should be noted that all histograms are coded, since the base lines represent 50° rather than 0° Lintner.

It is probably best to consider first the histogram in the lower right-hand corner, which presents the mean values for each variety over all stations and malting treatments. The varieties fall in the following order: Olli, 158° Lintner; O.A.C. 21, 142° L.; Hannchen, 125° L.; and Regal, 97° L.

By considering the five histograms in the last column we can now determine: (i) whether differences exist between the average values for each station; and (ii) whether the varieties fall in the same order at each station. Nappan samples gave the lowest average value, 105° L.; samples from Kapuskasing and Ottawa gave medium values of about the same order, 123° and 128° L.; and samples from Lacombe gave the highest average value, 165° L. More detailed comparisons of the five histograms will show that at Kapuskasing and Ottawa the varieties fell in the order shown by the means (black histogram); at Nappan the main discrepancy from this order is that Olli gives a lower value than O.A.C. 21, and at Lacombe the main discrepancy is that Hannchen gives a higher value than O.A.C. 21. It is thus apparent that the differential effect of stations (*i.e.*, environment) on varieties is very considerable.

The bottom row of histograms illustrates: (i) the mean differences between malting treatments; and (ii) the differential effect of treatments on varieties. It is apparent that diastatic activity was increased by increasing the germination time, by increasing the moisture content, and by raising the temperature. Since the configurations of all histograms in the bottom row are very similar, it is obvious that there was comparatively little interaction between treatments and varieties. It is apparent, however, that there was some interaction. For instance, in general, a change in treatment that tended to increase diastatic activity had a greater effect on Olli than on Regal.

The remaining 16 histograms (first four rows and first four columns) present the results for each treatment at each station. Comparison of these within rows shows the interaction between varieties and treatments within stations, and comparisons within columns show the interaction between varieties and stations within treatments. Since the histograms are very similar in configuration within rows, whereas considerable differences exist between the configurations of histograms in different rows, there can be no doubt that with respect to diastatic activity the differential effect of stations on varieties was considerably greater than the differential effect of malting treatments on varieties.

Wort Nitrogen

The family of histograms for total wort nitrogen as percentage of malt dry matter is shown in Fig. 2. These are arranged in exactly the same order as

those in Fig. 1. They are also coded: the base lines represent 0.65% rather than 0%.

The histogram with black columns shows that with respect to mean values over all stations and treatments, the varieties fall in the following order: Olli, 1.00%; O.A.C. 21, 0.89%; Hannchen, 0.87%; and Regal, 0.84%. The mean values for stations over all varieties and treatments are: Nappan, 0.80%; Kapuskasing, 0.86%; Ottawa, 1.00%; and Lacombe, 0.94%.

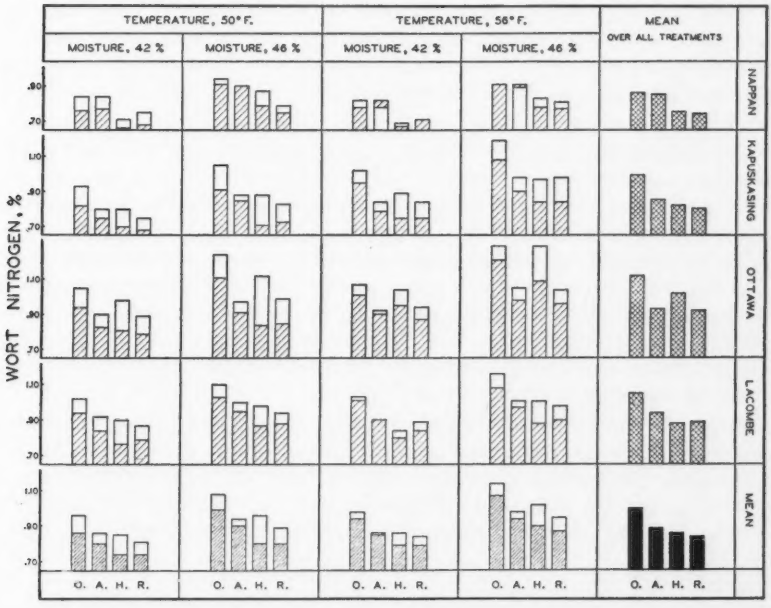

FIG. 2. *Histograms for wort nitrogen illustrating the differential effects of stations and malting treatments on varieties. For additional explanation see title of Fig. 1.*

The last column of histograms shows that the varieties fell in different orders at different stations. The configuration of the histogram for Kapuskasing is almost identical with that for the means. At Nappan, O.A.C. 21 gives as high a value as Olli; at Ottawa, Hannchen gives considerably higher values than O.A.C. 21 and Regal; and at Lacombe, Regal gives a slightly higher, rather than a lower, value than Hannchen. Thus with respect to wort nitrogen, it is apparent that environment has a considerable differential effect on varieties.

The differential effect of malting treatments on varieties is illustrated by the last row of histograms. If only the six-day malts are considered (hatched portions of columns only) it is apparent that the histograms have much the

same configurations. A fair degree of similarity also exists between the histograms for nine-day malts (tops of columns), although it will be observed that a change in moisture content has a considerable effect on configuration. The configurations of the histograms for the six- and nine-day malts differ considerably. It is thus apparent that the interaction between varieties and germination time is fairly large, and is greater than the interaction between varieties and moisture content, and that this again is greater than the interaction between varieties and germination temperature.

Further inspection of the histograms in the bottom row will show that the two-rowed variety Hannchen contributed most to the differential effect of treatments on varieties. It showed a far greater response to the increase in germination time than any of the other varieties. O.A.C. 21 also contributes, since it tends to respond least to changes in treatment.

By considering the remaining 16 histograms by rows, the interaction between varieties and treatments within stations can be elucidated. It will be observed that at Nappan, O.A.C. 21 samples grown at 56° F. gave a lower percentage of wort nitrogen after nine than after six days of germination. A similar tendency for the percentage nitrogen to remain constant or decrease slightly, with an excessive increase in germination time, was observed in a study of a low nitrogen sample of O.A.C. 21, reported in Part II of these studies (2). It will also be observed that at Ottawa, Hannchen showed a far greater increase in wort nitrogen with increasing germination time than it did at the other three stations.

A general comparison of the differences in the configurations of histograms within and between rows, special attention being paid to the last row and the last column, leaves little doubt that the differences between rows are greater and thus that the differential effect of stations on varieties is appreciably greater than the differential effect of malting treatments on varieties.

Extract

The coded histograms (base line, 67.5%) for extract are presented in Fig. 3.

The mean values for varieties over all stations and treatments are: Olli, 77.6; O.A.C. 21, 75.6; Hannchen, 78.0; and Regal, 72.9.%. The mean values for stations, over all varieties and treatments, are: Nappan, 77.9; Kapuskasing, 76.3; Ottawa, 75.8; and Lacombe, 74.3%.

The last column of histograms again shows that the differential effect of stations on varieties is quite large. Hannchen is about equal to Olli at Nappan, appreciably higher at Kapuskasing and Ottawa, and appreciably lower at Lacombe. These two varieties yield considerably more extract than O.A.C. 21 except at Lacombe, where Hannchen is only slightly above O.A.C.21. Regal yields the lowest value at all stations but is not much lower than O.A.C. 21 at Kapuskasing.

The differences in the effects of treatments are particularly obvious in this family of histograms. At the higher temperature and moisture level (fourth

column), the nine-day malts yielded less extract than the six-day malts, whereas with other treatments the reverse holds true. It is thus apparent that the malts made at the higher temperature and moisture level were very much overgrown at nine days, with the result that the extract yield was decreased by excessive malting loss. Results of this type were also obtained in the investigation reported in Part II of this series (5) and are there illustrated by curves in which extract yield is plotted against germination time.

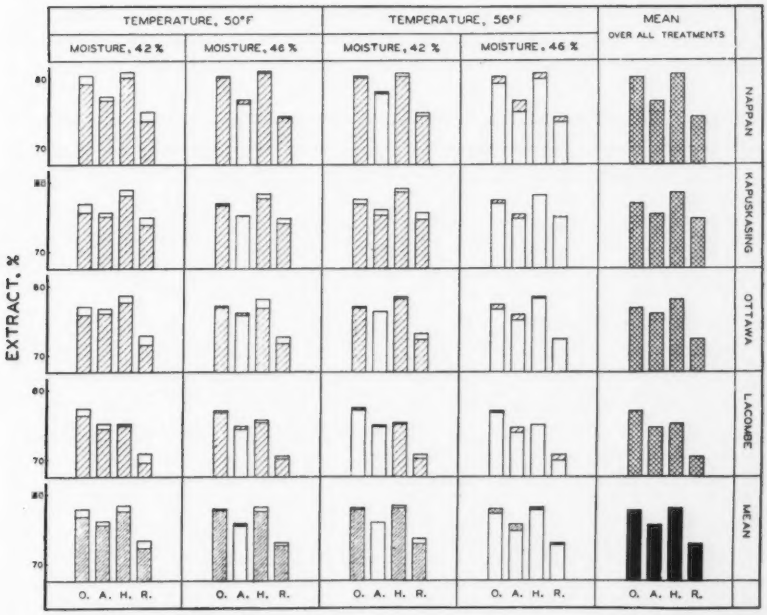

FIG. 3. *Histograms for extract illustrating the differential effects of stations and malting treatments on varieties. For additional explanation see title of Fig. 1.*

Inspection of the individual rows of histograms will show that there is an appreciable interaction between varieties and malting treatments. This is particularly noticeable with respect to the differences between six- and nine-day malts made at 50° F. and 46% moisture. At three out of four stations, O.A.C. 21 yielded less extract at nine than at six days, whereas the other varieties, particularly Hannchen and Regal, yielded more extract at nine than at six days, at all stations. The results for 56° F. and 46% moisture are similar, since the decrease in extract from six to nine days is greater for O.A.C. 21 than for any of the other varieties.

In general it is quite apparent that the differences between the configurations of histograms within rows are much less than the differences between the

configurations of histograms in different rows. It follows that with respect to extract, the differential effect of malting treatments on varieties was much lower than the differential effect of stations on varieties.

Malting Loss

The coded histograms (base line at 5%) for malting loss are presented in Fig. 4.

The mean values for varieties, over all stations and malting treatments, are: Olli, 10.8; O.A.C. 21, 10.7; Hannchen, 11.1; and Regal, 10.1%. The mean values for stations, over all varieties and treatments, are: Nappan, 10.1; Kapuskasing, 10.4; Ottawa, 10.7; and Lacombe, 11.6%.

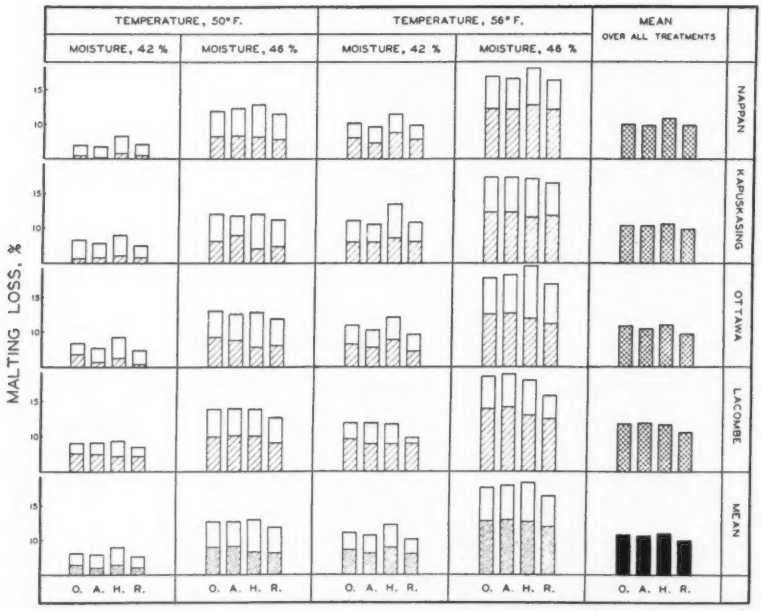

FIG. 4. *Histograms for malting loss illustrating the differential effects of stations and malting treatments on varieties. For additional explanation see title of Fig. 1.*

Inspection of the last column of histograms will show that there was some interaction between stations and varieties. The relations between Hannchen and Regal remain about the same at all stations. On the other hand, by comparison with Hannchen, Olli and O.A.C. 21 give much lower values for Nappan and slightly higher values for Lacombe.

The bottom row of histograms again illustrates the differential effect of treatments on varieties. Considering the six-day malts only, it is apparent

that at the lower moisture levels (first and third columns of histograms) O.A.C. 21 gives lower values than Olli or Hannchen, whereas at higher moisture contents (second and fourth columns) O.A.C. 21 gives higher values. Again considering the six- and nine-day malts, it is apparent that the additional three days' growth resulted in a greater increase in the malting loss for Hannchen than in the malting losses for the other three varieties.

In general, the differences between the configurations of histograms within rows are of about the same order as the differences between rows. It accordingly appears that with respect to malting loss, the differential effect of malting treatments on varieties is of about the same magnitude as the differential effect of stations on varieties.

Interactions Between Germination Time, Temperature, and Moisture Level

The investigation also brought to light certain interactions between germination time, temperature, and moisture level. These effects cannot be readily elucidated by study of the histograms in Figs. 1 to 4 and data illustrating them are therefore given in Table I. The data presented are mean values for all samples.

TABLE I

DATA FOR DIASTATIC ACTIVITY, WORT NITROGEN, EXTRACT, AND MALTING LOSS, ILLUSTRATING INTERACTIONS BETWEEN GERMINATION TIME, TEMPERATURE, AND MOISTURE LEVEL

Property	Time × temperature			Time × moisture			Moisture × temperature		
	Time	Temperature		Time	Moisture		Moisture	Temperature	
		50° F.	56° F.		42%	46%		50° F.	56° F.
Diastatic activity	9 days	133	143	9 days	131	145	46%	130	144
	5 days	116	130	6 days	117	130	42%	119	129
	Difference	19	13	Difference	14	15	Difference	11	15
Wort nitrogen	9 days	.893	.956	9 days	.909	.996	46%	.866	.984
	6 days	.829	.919	6 days	.813	.880	42%	.827	.921
	Difference	.064	.037	Difference	.096	.116	Difference	.039	.063
Extract	9 days	76.3	76.0	9 days	76.5	75.9	46%	76.0	75.9
	6 days	75.7	76.1	6 days	75.8	76.0	42%	76.0	76.3
	Difference	0.6	−0.1	Difference	0.7	−0.1	Difference	0	−0.4
Malting loss	9 days	10.4	14.4	9 days	9.6	15.1	46%	10.6	15.1
	6 days	7.4	10.6	6 days	7.3	10.6	44%	7.2	9.8
	Difference	3.0	3.8	Difference	2.3	4.5	Difference	3.4	5.3

The interaction between time and temperature was quite marked with respect to each of the four properties studied. A three-day increase in germination time has a greater effect at 50° than at 56° F. on diastatic activity, wort nitrogen and extract, but the effect on malting loss is less at 50° than at 56° F.

The data also show that interactions existed between time and moisture, and moisture and temperature.

The data for extract are particularly interesting. It will be observed that at the lower temperature and at the lower moisture level, extract increased with an increase in germination time from six to nine days, whereas at the higher temperature and moisture level, extract decreased with increasing germination time. Similarly, a decrease in extract also took place at 56° F. when the moisture content was raised from 42 to 46%, whereas at 50° F. the change in moisture had no effect on extract yield.

The existence of these interaction effects emphasizes the difficulty of making accurate statements about the general effect on malting quality of a change in malting conditions. The effect of a change in any one malting factor such as germination time will depend upon the levels at which other factors such as temperature and moisture are maintained. Moreover, it will also depend upon the variety and total nitrogen content of the barley under investigation.

Statistical Analyses

As a first step in the statistical analyses, the variance of the data for each determination was analysed into portions due to: (i) average differences between varieties; (ii) average differences between stations; (iii) average differences between malting treatments; (iv) differences in the relative average performance of varieties at different stations; (v) differences in the relative average performance of varieties under different treatments; (vi) differences in the relative average performance of stations under different treatments; (vii) the second order interaction between varieties, stations and treatments; and (viii) differences between duplicate malts (for diastatic activity and malting loss only). The results of these analyses are reported in Table II.

TABLE II

ANALYSES OF VARIANCE FOR DIASTATIC ACTIVITY, WORT NITROGEN, EXTRACT, AND MALTING LOSS

No.	Variance due to	Degrees of freedom	Mean squares			
			Diastatic activity	Wort nitrogen	Extract	Malting loss
i	Varieties	3	42,986**	.1697**	175.18**	12.63**
ii	Stations	3	40,843**	.2275**	70.84**	26.20**
iii	Treatments	7	4,947††	.0924††	1.87††	416.567††
iv	Varieties × stations	9	1,347‡‡	.0123‡‡	8.41‡‡	1.33‡
v	Varieties × treatments	21	76**	.0022**	.26**	1.20**
vi	Stations × treatments	21	58**	.0049**	.18**	1.18**
vii	Varieties × stations × treatments	63	18	.0007	.05	.28
viii	Duplicate malts	128	6	–	–	.38

***Significantly greater than* (iv); ††*Significantly greater than the larger of* (v) *and* (vi); ‡*Significantly greater than the larger of* (vii) *and* (viii).

NOTE: *In this and the following table, double signs denote that the 1% level, and single signs that the 5% level of significance is attained.*

The statistics show that, with respect to each determination, the mean squares for varieties and stations are significantly greater than the mean square for the interaction between these two factors; that the mean square for malting treatments is significantly greater than the mean squares for the interactions between this factor and stations or varieties; and that the mean squares for the interactions between each pair of the three main factors are significantly greater than the mean square for the second order interaction between the three factors, which forms an estimate of the error of the investigation.

As the investigation was undertaken with the main object of comparing the interaction between varieties and stations with the interaction between varieties and treatments, particular attention should be paid to the corresponding mean squares. The ratios of the mean squares for varieties × stations to the corresponding mean squares for varieties × treatments are as follows: for diastatic activity, 17.7; for wort nitrogen, 5.6; for extract, 32.6; and for malting loss, 1.1. It is thus apparent that, except with respect to malting loss, the differential effect of stations on varieties is a great deal larger than the differential effect of malting treatments on varieties.

As a further step in the analyses, the variance due to the interaction between varieties and malting treatments was analysed into portions due to: (i) differences in the relative performance of the varieties with different germination times; (ii) differences in the relative performance of the varieties at different moisture levels; (iii) differences in the relative performance of varieties at different temperatures; and (iv) a remainder, representing the second and third order interactions, which forms an appropriate estimate of error. The results of these analyses are given in Table III.

TABLE III

ANALYSES OF VARIANCE DUE TO INTERACTION BETWEEN VARIETIES AND MALTING TREATMENTS

No.	Variance due to	Degrees of freedom	Mean squares			
			Diastatic activity	Wort nitrogen	Extract	Malting loss
i	Varieties × times	3	170‡‡	.00843‡‡	.587‡‡	3.77‡‡
ii	Varieties × moisture	3	179**	.00387**	.464*	3.30**
iii	Varieties × temperatures	3	104*	.00120	.361*	1.10**
iv	Remainder	12	19	.00046	.097	.06

** and ‡‡ significantly greater than remainder mean square.*

Of the three interactions studied, that between varieties and temperatures is lowest for all determinations. For wort nitrogen the interaction between varieties and times is highest, but for the other three determinations the interactions between varieties and times and between varieties and moisture levels are of much the same magnitude.

Discussion

The results of the investigation are quite clear-cut. The varieties did not fall in exactly the same order, with respect to any determination, when they were grown at different stations. It is thus clear that environment had a marked differential effect on varieties. Moreover, it is also apparent that when the malting treatments were changed, the relative positions of the varieties, with respect to each determination, were also appreciably changed. However, except with respect to malting loss, this differential effect of malting treatments on varieties was by no means as great as the differential effect of environment on varieties. The latter was thus the limiting factor in the study of the varieties, but in spite of it, significant differences were demonstrated between varietal means for each determination.

These conclusions apply specifically to the particular varieties, stations, and malting methods studied in the present investigation. Because of the small numbers of samples and methods, and because these do not represent *random* selections from larger homogeneous populations, there is some danger in arguing from the particular case reported in this paper to a more general one. It will be borne in mind, however, that by exaggerating the differential effect of malting treatment on varieties, but not the differential effect of environment on varieties, a deliberate attempt was made to disprove the hypothesis that the latter is greater than the former. Considerable weight must therefore be attached to the fact that in spite of this attempt, the results for diastatic activity, wort nitrogen, and extract provide strong support for the hypothesis.

With respect to malting loss the two differential effects were of the same order. This serves to emphasize the necessity for caution in interpreting data on this property. The matter has always been a difficult one. Differences between varieties are generally small by comparison with the precision with which they can be determined, though significant differences between certain pairs of varieties can frequently be demonstrated in spite of this limitation. It seems difficult to estimate the importance and significance of varietal differences in malting loss as determined under standardized conditions, and the results of the present investigation merely serve to increase the difficulties and emphasize the need for further research.

In Canada, the routine laboratory malting test is used for comparing newly developed or newly introduced varieties with the standard variety, O.A.C. 21. The test varieties and the standard are grown in small plots at a number of stations. The barleys are analysed, malted under standard conditions, and the malts are also analysed. Since the barley varieties do not fall in exactly the same relative positions at all stations, with respect to any barley or malt property (*i.e.*, since there is a differential effect of environment on varieties), the mean difference between a test variety and the standard must attain a certain magnitude before it can be considered significant. It has been our experience that fairly reliable estimates of differences in malting quality can be obtained by growing the varieties at twelve widely separated experimental

stations. Under these conditions, mean differences of about 1% in extract, 10% in diastatic activity, and 10% in wort nitrogen, generally prove to be significant. In other words, if differences of this order are found we should expect more reliable results, obtained by testing a far greater number of samples, to show conclusively that real differences exist between the varieties.

The conditions used in the routine test simulate those used in commercial plants in Canada and are thus adapted for malting average samples of the standard variety, O.A.C. 21. The question that now arises is whether a test variety might not show up to better advantage if the malting conditions were changed. As a result of this and earlier investigations (2, 3, 5), it appears that the spreads between certain pairs of varieties can be affected by changing the malting conditions. This is particularly true of varieties that differ widely in malting characteristics, but if wide differences are shown to exist between any pair of varieties under one set of malting conditions, it seems probable that these will persist under all sets of conditions within a reasonable range. In general, we believe that if the routine test shows that, on the average, a variety differs from the standard by more than 1% in extract, or 10% in diastatic activity or wort nitrogen, it will be found that a real difference exists between the varieties, which cannot be overcome by any reasonable change in malting conditions. The routine test should therefore serve for the elimination of varieties that are definitely inferior to the standard with respect to one or more important malt qualities. On the other hand, all varieties that the routine test shows to be reasonably promising will require further and more extensive study before it is possible to decide whether they are equal in malting quality to O.A.C. 21.

Acknowledgments

The authors are indebted to Mr. P. R. Cowan, of the Central Experimental Farm, Ottawa, and to a number of other members of the staff of the Dominion Experimental Farms Service, for their co-operation in providing large samples of the required barley varieties.

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THE ACCURACY OF THE PLATING METHOD FOR ESTIMATING THE NUMBERS OF SOIL BACTERIA, ACTINOMYCES, AND FUNGI IN THE DILUTION PLATED[1]

By Norman James[2] and Marjorie L. Sutherland[3]

Abstract

During the crop seasons of 1936, 1937, and 1938, 1465 samples of field soil, held in the laboratory one day after crushing, were plated by the recognized technique in four replicates of one dilution for counts of fungi, and of a higher dilution for counts of bacteria and of actinomyces. A χ^2 value was calculated for each set of counts. These values for each group of micro-organisms were distributed into classes, and the number in each class was compared with the theoretical for the Poisson series. The data for each year indicate that the fungal counts conform to expectancy on the basis of random sampling, and show that the method provides a reasonably accurate estimate of the population in the dilution plated capable of developing under the conditions of growth. Too many sets of counts of bacteria in each year yield high χ^2 values. The counts of actinomyces conform to expectancy.

In an attempt to determine the cause of this abnormality for sets of counts of bacteria, samples were plated on the afternoon of the day they were taken from the field. Three hundred and four samples plated in six replicates of one dilution, and another 100 samples plated in four replicates, yield χ^2 values whose distributions conform to expectancy. Accordingly, the plate method provides a satisfactory estimate of the bacterial population of soil in the dilution plated if the procedure is carried out within six hours after sampling. Data on 88 samples plated on the day of sampling, on 88 samples held one day, on 88 samples held two to five days, and on 88 samples held eight to thirteen days show that the discrepancy between the actual and theoretical distributions of χ^2 values becomes progressively greater at each successive period of holding the samples. Further, the data indicate that the area sampled, the season, the medium used and the technique of plating bear no relation to the abnormal variation in counts of bacteria on replicate plates.

A record was kept of the presence of abnormal types of bacterial colonies and various genera of fungi on all plates from 468 samples plated one day after sampling and crushing during 1938. The data show that sets having pin-point colonies or spreading colonies of the Mucorales on one or more plates usually have high χ^2 values. Counts on such plates should be excluded from the estimate of the mean number of bacteria in the sample. Likewise, the number of actinomyces colonies on each plate from these samples was recorded. The χ^2 values for these counts were found to conform to expectancy, indicating that the factor or factors associated with a large number of high χ^2 values for counts of bacteria does not affect the count of actinomyces in the same way.

Percentage moisture and P values corresponding to χ^2 values for the counts of bacteria obtained each year were correlated. The data yield coefficients that are not significant in each case.

Introduction

The problem of estimating the numbers of micro-organisms in soil has engaged the attention of soil microbiologists since the beginning of the science and, after a half century marked by methods that are far from adequate, still has a fascinating appeal. The soil is known to be teeming with micro-

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scopic life. There must be some way of estimating with reasonable accuracy the number of the various forms that exist in a given sample. The plate method, because of its wide use in early studies and because of its association with counts in other substances, particularly dairy products, still offers promise of providing results that have practical application. It is not without its limitations, which are recognized and cannot be considered lightly. The statement by Conn (5) that "It has in fact long been realized, even by those using these methods, that such counts have little significance; and they have come to be used less and less as bacteriologists have learned more about bacteria in the soil" represents an opinion that scarcely can be accepted as final. This investigator, supported in his opinion by Winogradsky (36, 37), favours the direct microscopic technique, which to date has not been given general approval and has not provided evidence of superiority (25). Thornton and Gray's (25) belief, in reference to the plating method, that "such counts have undoubted value in comparing two or more samples" appears to be shared by many who have continued to use the plating method, even since the development of the direct method. Among the foremost of these may be listed the following: Bisby *et al.* (1), Brierley *et al.* (2), Brown and Benton (3), Cobb (4), Corbet (6), Eggleton (7), Erdman (8), Gray *et al.* (14, 15), Harmsen and Verweel (16), Jensen (17), Lochhead and Thexton (18), Newton (19), Stevens (20), Taylor (22), Taylor and Lochhead (23), Timonin (24), Vandecaveye *et al.* (26–28), Waksman and Purvis (32), Williams (33), Wilson and Lyon (34), and Wilson and Kuhlmann (35).

Historical

The mathematical analysis of data obtained by the plating technique has engaged the attention of many investigators. Fisher *et al.* (9) developed a statistical control method for checking short series of parallel plates. They showed that, under ideal conditions of plating, the counts of bacterial colonies on parallel plates vary in the same manner as samples from the Poisson series; and that, when these conditions are fulfilled, the mean count of replicates is a direct measure of the density of the population. For a large number of sets of parallel plates, agreement with the theoretical distribution may be tested by

$$\chi^2 = \frac{S(x - \bar{x})^2}{\bar{x}}$$

where x is any number of colonies counted on a plate and $\bar{x}$ is the mean count of the replicates.

Waksman (29) found a probable error of 1.8% in counts of total bacteria, 2.7% in counts of actinomyces, and 5.8% in counts of fungi, when considering 50 plates from each plot for each group of micro-organisms. He presented, in addition, a table giving a weighted value based upon the number of samples from one source and the number of plates from the sample. To obtain a weight of 3.00, or the minimum for a very good rating, would require ten plates from each of three samples or two plates from each of ten samples.

Wilson and Kuhlmann (35) applied the χ^2 method to counts of rhizobia on Fred and Waksman's medium 79 (12) obtained by a standardized technique, and found that in data based on sets of three replicate plates the large values of χ^2 arose from counts in which one plate showed undue variation from the other two. In a second experiment in which five replicates were used, either the plate that showed marked deviation from the others, or the third plate when there was close agreement in all, was eliminated. In this case, the variations noted in the remaining plates could have arisen by chance alone.

Gray and associate (14, 15) prepared five replicates from each sample, but in some cases the count could be calculated from a smaller number of plates only. They show the number of plates used and the reliability of the results in relation to the χ^2 index of dispersion.

Jensen (17) applied statistical tests to counts of bacteria, actinomyces, and fungi in fifty samples of soil. He used formulae designed to test the variation when the number of replicate plates is not constant. The method does not verify the theoretical distribution with any exactitude but does test whether the general level of variability conforms with expectation (10). This investigator found the variation between parallel plates of bacteria to be very close to the expected, while that for replications of actinomyces showed a tendency to subnormality. He suggests that, since in the case of the actinomyces the difference is not very great, there is justification in considering the majority of the actinomyces counts as reliable. The test applied to the counts of fungi gave a difference within the permissible limits and he concludes that the technique must be considered as giving a reliable index of the numbers of mycelial fragments and fungal spores capable of developing on the medium used.

Harmsen and Verweel (16) applied Fisher's control test to a large number of series of replicate counts and presented histograms which indicate that numbers of bacteria found on parallel plates from field samples have an abnormal variance. This is proved true also on data published by Waksman and submitted to the test by these investigators. In this work, and in that of Waksman also, ten replicate plates from each sample were used. However, they found a very satisfactory distribution of χ^2 values on the data from actinomyces.

In a recent paper by Sutherland and James (21), the literature dealing with the problem of the application of the χ^2 test to bacterial counts by the plate method was reviewed. In this report it is shown that the technique of making dilutions and preparing plates produces a mean of four counts that is accurate as an estimate of the population in the dilution sampled when certain pure cultures are used. This finding is of interest from the standpoint of eliminating technique as the cause of the discrepancies in the χ^2 distributions referred to in previous reports.

Experimental

Scope of the Problem

The procedure for estimating the number of micro-organisms in the soil of a given field involves so many steps that there is probability of serious error. The work reported hereafter is an attempt to determine the accuracy of each step in the laboratory by the application of accepted mathematical tests to the data obtained and, where necessary, to provide a technique that is satisfactory for the purpose intended. Obviously, this necessitates starting at a point farthest from the field sample and progressing one step at a time. Accordingly, this presentation deals only with the accuracy of the mean count of four or six replicates from one dilution as an estimate of the population in the dilution plated.

A second paper will deal with the accuracy of the count from one dilution as an estimate of the population in the aliquot sample used in making the dilution, and the accuracy of the count from one aliquot sample as an estimate of the population in the sample brought to the laboratory.

I. The Accuracy of the Mean Count of Four Replicate Plates from One Dilution: Bacteria

Method of Procedure

The 1465 samples used in this study were taken from two permanent series of plots in the experimental field of the Dominion Rust Research Laboratory at the University of Manitoba, in a long-term investigation of the microflora of grain-producing soils in relation to the cereal root-rot problem. The soil is a dark brown, heavy clay that becomes compact and sticky when wet. At many samplings the soil had a moisture content of over 40% and did not break up readily. Each laboratory sample was a composite of six cores, 6 by $1\frac{1}{4}$ in., taken at random from a hundredth-acre plot. It was crushed by careful hand manipulation and mixed thoroughly. A moisture test was run, and sufficient soil to give 25 gm. on a dry basis was added to 240 ml. of sterile water in a pint jar. This dilution was shaken for five minutes on a mechanical rocker shaker and given a vigorous shaking by hand immediately before a transfer was made. Higher dilutions were made in six-ounce, screw-topped medicine bottles and shaken by hand 25 times. The 1 : 200,000 dilution was used for plating in 1936. This was changed to 1 : 800,000 in 1937 and 1 : 500,000 in 1938. The higher dilutions seemed advisable from the standpoint of ease in counting. The number of colonies per plate ranged from 40 to 200, except in occasional samples where pin-point colonies raised the count. A one-millilitre sterile pipette was rinsed once in the dilution to be plated and one ml. was delivered to each of four plates.

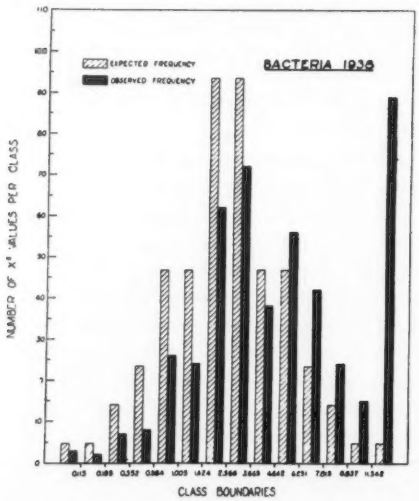
Fred and Waksman's (12) sodium albuminate agar was used for bacteria and actinomyces. This medium has been used by Harmsen and Verweel (16) in a similar study, and by others. Incubation was at 25 to 28° C. for eight days. The higher temperature appeared necessary because of the impracticability

of maintaining a lower temperature during the excessive heat of midsummer. A mounted lens of four-inch diameter and two to three diameters magnification was used to check all plates before recording the counts.

Mean and χ^2 (Chi square) values were calculated for each set of counts on four replicates. The χ^2 values were distributed into classes on the basis of class boundaries for three degrees of freedom. Finally, the goodness-of-fit test (13) was used to ascertain the agreement between the actual and expected or theoretical distributions.

Results

The goodness-of-fit test applied to the distribution of χ^2 values for counts of bacteria on 504 samples investigated in 1936 gives a final χ^2 value of 1336.08, with a P value decidedly below the 1% point. This finding was confirmed on 493 samples studied in 1937, and on 468 samples in 1938. The data for 1938 are presented graphically in Histogram 1.



Histogram 1. *χ^2 distribution on 468 samples for counts of bacteria, 1938.*

These results show that the distributions of χ^2 values vary widely from expectancy. They point clearly to some factor or factors responsible for the exceedingly small P values in the data presented. A large series of sets of parallel plates should yield χ^2 values that are distributed according to the Poisson series, if the technique of dilution affords a perfectly random distribution of organisms, and if these can develop on the plate without mutual interference (10). A P value between 0.9 and 0.1 may be accepted as indicating that the variation among counts on replicate plates from one dilution may be attributed to chance.

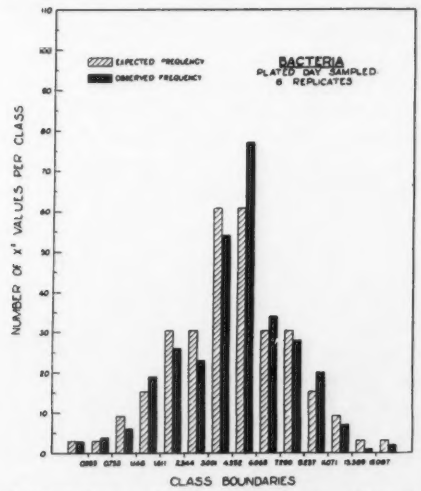
II. Factors Contributing to the Abnormal Variation Among Counts of Bacteria on Replicate Plates from One Dilution

(a) Time Sample Held in the Laboratory before Plating

The data obtained in the preceding section were obtained on samples held in the laboratory one day after they were broken up. This was carried on as a routine procedure to allow time for moisture determinations in order to obtain counts directly on the basis of the amount calculated to make 25 gm. of dry soil. Late in the summer of 1938 this procedure proved to be faulty from the standpoint of abnormal findings in the tests of variance to be considered in a later paper. As a consequence, data on 88 samples plated on the afternoon of the day of sampling were available for χ^2 distribution studies. The failure to find the usual abnormal distribution of χ^2 values prompted the continuance of the study of fresh samples as they affect the accuracy of the mean of four or six replicates from one dilution in conjunction with the tests of variance studies referred to above. Unfortunately, the record of how long others have held samples before plating is not made clear. The work of Harmsen and Verweel (16) gives no indication of their procedure in this respect. Likewise, the data furnished by Waksman (31) and analysed by these investigators, give no definite information on this point. Waksman (30) states that the samples were "plated as soon as possible", which allows for varied interpretations.

Results

The distribution of χ^2 values on 304 samples, each plated in six replicates from one dilution, is shown in Histogram 2. The goodness-of-fit test applied



Histogram 2. *χ^2 distribution on 304 samples plated immediately for counts of bacteria, six replicates.*

to this distribution gives a final χ^2 value of 14.19 with a corresponding P value of approximately 0.36. This finding was confirmed on 100 samples plated in four replicates, which yielded a final χ^2 value of 7.82 with a corresponding P value of about 0.85.

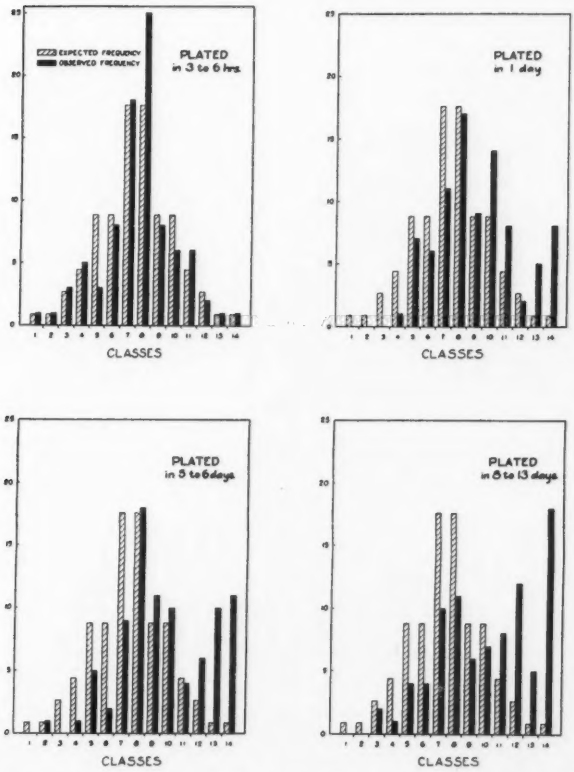
These results show that the distributions of χ^2 values conform to expectancy, and indicate clearly that the discrepancy in the distribution of χ^2 values resulting from abnormal variation among counts of replicate plates from one dilution may be avoided by proceeding with the laboratory technique of diluting and plating within a few hours after sampling.

Further evidence on the effects of holding samples of soil in the laboratory on the abnormality of the distribution of χ^2 values is shown in the data on four series of counts on samples held for different periods. In this experiment the samples were not plated primarily to study the distribution of χ^2 values, and consequently the counts at the various ages are not on the same samples. However, they represent samples taken from the same field, plated by the same technique, covering the same period of the year (July 21 to Sept. 1, 1938), and may be considered comparable. Each series is on 88 samples. The first series represents samples plated in six replicates from one dilution on the afternoon of the day obtained; the second, plated in four replicates one day after breaking up; the third, in six replicates after holding five to six days; and the fourth, likewise in six replicates after eight to thirteen days. Since for given P values the χ^2 values for the class boundaries differ with the degrees of freedom, the data in the goodness-of-fit test are presented on the basis of the P value distribution, but with arbitrary numbers designating classes.

The distributions for these 352 samples are presented in Histogram 3. The goodness-of-fit test gives final χ^2 and P values of 9.62 and 0.73 for the samples plated within three to six hours; 93.86 and less than 0.01 for the samples plated after holding one day; 233.19 and less than 0.01 for the samples held five to six days; and 405.28 and less than 0.01 for the samples held eight to thirteen days. The gradual rising of the final χ^2 values indicates a progressive increase in the degree of abnormality and suggests that the factors responsible become more marked as the time the samples are held in the laboratory is prolonged.

(b) Certain Types of Bacteria and Fungi on Plates for Bacterial Counts

During the first two years of this study, the abnormal count on one or more plates in a set frequently appeared to be associated with the presence of certain types of bacteria or fungi on the plates. In an attempt to determine whether these types of organisms are associated with the abnormal distribution of χ^2 values, their presence was recorded on the plates prepared in 1938. This was done on 468 samples, representing 1872 plates. The identification and recording of these types were carried out before the plates were counted, in order not to look for their presence only on plates with low counts. These were considered in relation to the distribution of χ^2 values.



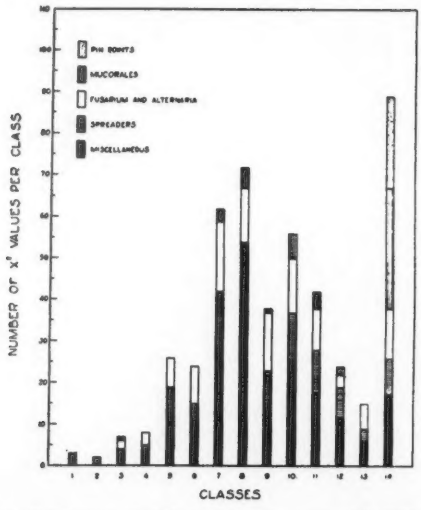
HISTOGRAM 3. *χ^2 distribution on 352 samples held varying times for counts of bacteria.*

RESULTS

The presence of an excess of pin-point colonies was noted on one or more plates from 23 samples; of these, 22 had χ^2 values with a P value lower than 0.01. Cultures of the order Mucorales occurred 51 times. Most of these were *Rhizopus nigricans*, but not all. Of these, 29 sets had abnormally high χ^2 values. *Fusarium* spp. and *Alternaria* spp. appeared in 109 samples. These were spread over the χ^2 class value range. The same was true of spreader types of bacterial colonies, which were recorded on 91 sets. The remaining 194 had colonies of various genera, including *Penicillium*, *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Cylindrocarpon*, *Monotospora*, *Phoma*, *Trichoderma*, *Verticillium* and others occurring rarely which did not appear in one class more often than in another, or were free from unusual colonies.

The data are presented graphically in Histogram 4. In order to simplify the presentation of these data, the term "miscellaneous" included the 194 sets

mentioned above; and *Fusarium* spp. and *Alternaria* spp. were grouped together. Further, as plates in one set frequently had more than one type of colony recorded, they were distributed arbitrarily in classes in the following order of preference; pin-points, *Mucorales*, *Fusarium* and *Alternaria*, spreaders and miscellaneous. For example, a set with a *Mucorales* and a *Penicillium* was placed in the *Mucorales* class only.



HISTOGRAM 4. *χ^2 distribution on 468 samples for counts of bacteria, 1938, showing the effect of certain types of bacteria and fungi.*

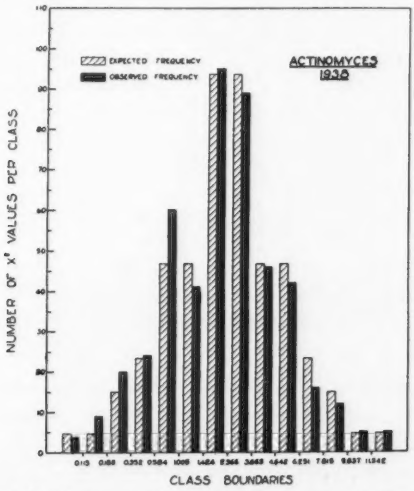
These findings indicate that there is sound reason for rejecting plates that have a large number of pin-point colonies or are overgrown with *Rhizopus nigricans*, particularly if the plates concerned differ in count appreciably from others in the set. Had this been done in this analysis, the number of sets having abnormally high χ^2 values would have been reduced more than one-half. There still remain too many sets with high χ^2 values, the cause of which is not suggested.

(c) The Presence of Actinomyces on Plates for Bacterial Counts

The counts reported as bacterial counts in this presentation include the actinomyces group. Since actinomyces may be distinguished from bacterial colonies, an attempt was made to determine whether the discrepancy in the distribution of χ^2 values for the total count was characteristic of both groups. During 1937, actinomyces were counted after incubation for 12 days. The counts in 1938 were made at eight days at the time of making the total counts, so as to provide three sets of data for each plate, namely, the total count, bacteria only, and actinomyces.

Results

The goodness-of-fit test applied to the distribution on 483 samples investigated in 1937 gives a final χ^2 value of 32.21 and a P value slightly below 0.01. The 468 samples studied in 1938 give final χ^2 and P values of 14.51 and 0.34, respectively. The finding for 1938 appears in Histogram 5.



HISTOGRAM 5. *χ^2 distribution on 468 samples for counts of actinomyces, 1938.*

The counts of bacteria only, or the total count less the actinomyces, give a distribution with a final χ^2 value of 1927.43.

These results indicate that the discrepancy in the distribution of χ^2 values for the total count is due to variation among numbers of bacterial colonies rather than among numbers of the actinomyces. The factors causing the discrepancy influence the development of bacteria on certain plates, but apparently do not affect the actinomyces growth on this medium.

(d) Moisture Content of Sample Plated

Moisture tests were run on 1476 samples before plating. These varied appreciably, even among samples obtained at one time from adjoining plots. As moisture content might influence the factor or factors inducing the abnormal variation among counts on samples held in the laboratory, percentage moisture was correlated with P values corresponding to the χ^2 values obtained. Moisture readings were classed on a unit basis from 13 to 49. The P values were divided into 20 classes with an interval of 0.05 ranging from a P of 1.00 to a P of 0.00. Since the χ^2 values for all the P values required were not given in Fisher's table (11), and interpolation on a linear basis did not appear to be accurate, the figures given in this table were graphed on squared paper

on a scale large enough that the χ^2 and P values could be read to the second decimal. The χ^2 values for the missing P values were taken from the graph. A correlation surface for percentage moisture and χ^2 values was prepared from the data for each year and a correlation coefficient calculated for each.

Results

The data for 1936 give a correlation coefficient of 0.0836; for 1937, a coefficient of 0.0444; and for 1938, of 0.0697. The t test applied to the correlation coefficients gives t values of 1.87, 0.996, and 1.51 respectively. When n is taken as infinity the 5% level of significance has a t value of 1.96, and the 1%, a value of 2.58. These results may be accepted as indicating the absence of a significant correlation between moisture and χ^2 values. Consequently, moisture does not influence the distribution of χ^2 values obtained from sets of counts of bacteria on replicate plates from one dilution of soil.

III. The Accuracy of the Mean Count of Four Replicate Plates from One Dilution: Fungi

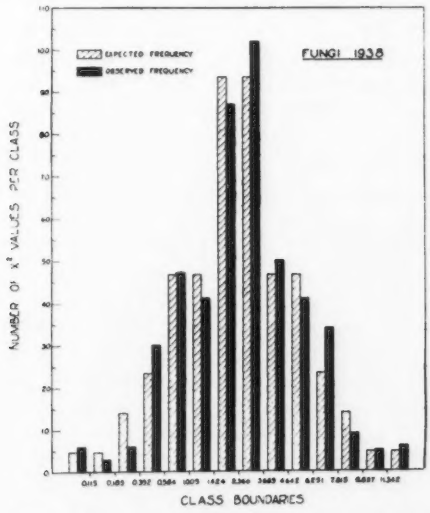
Method of Procedure

The samples referred to in Section I were plated for fungi at the same time as for bacteria. The 1 : 5000 dilution was used on all samples. The number of colonies ranged from 5 to 50, with most of the counts midway between these limits. Czapek's (12) medium was used. It was acidified by the addition of 0.5 ml. of lactic acid (10 ml. of 85% U.S.P.X. made to 100 ml. with distilled water) to 100 ml. of medium after the final tempering. Counts were made after four days' incubation at 25 to 28° C. The data were submitted to the mathematical tests applied to the bacterial counts.

Results

The goodness-of-fit test on the χ^2 distribution for counts of fungi on 468 samples investigated in 1936 gives a final χ^2 value of 32.32 and a P value slightly below 0.01. A better fit was observed on 498 samples plated in 1937; and on 467 samples in 1938. These two P values are 0.63 and 0.18. The finding for 1938 is shown in Histogram 6.

These results show a close agreement between the actual and expected distribution of χ^2 values and indicate that the mean count of four replicate plates may be used to estimate the number of fungi in the dilution plated. This is a confirmation of the finding of Jensen (17), but on a larger number of samples. It is recognized that the count may represent spores and pieces of mycelium and that the technique does not distinguish between them. Nevertheless, there should not be serious objection to the use of data obtained by this technique in comparing the numbers of potential fungi in different dilutions.



HISTOGRAM 6. *χ^2 distribution on 467 samples for counts of fungi, 1938.*

Discussion

The application of proven mathematical formulae to test the accuracy of data obtained by the soil bacteriologist requires little justification. The laboriousness of the technique involved in obtaining counts of micro-organisms by any method demands the use of every test that will verify the validity of the result or lessen the routine labour of the undertaking. The bacteriologist who makes an estimate of the numbers of fungi or bacteria in soil, does so for the purpose of relating the estimate to some condition or treatment. If his estimate is accurate, some practical advance may be made in an understanding of the intricacies of the complex medium with which he deals. If it is not, his findings retard rather than advance the science.

The most important result concerning the distribution of χ^2 values for sets of counts of bacteria has to do with the time the sample is held in the laboratory before plating. A sample plated on the day it is taken from the field yields counts that may be accepted as satisfactory for the dilution plated. The counts on samples held one day or more give χ^2 values that have abnormal distributions, similar to those presented by Harmsen and Verweel (16), who did not make clear how long their samples were held.

There still remains to be explained the cause of the abnormal distributions of χ^2 values from samples that are held before plating. Two conditions appear to be associated with an excess of high χ^2 values: a large number of pin-point colonies on one or more plates in a set, and large spreading colonies of the *Mucorales* on one or more plates. In this connection, the data presented

justify the exclusion of counts from such plates in a set. On the other hand, there is ample evidence in the data presented that certain factors bear no relation to the problem. The samples providing the good fit in the distribution of χ^2 values were obtained from the same plots as those that give the abnormal distribution. They were plated by the same technique, pertaining to diluting, pipetting, pouring, incubating, and counting, and by the same operators. The medium was the same. The counts giving a good fit were obtained over part of the same season as those that did not. This would appear to eliminate the source of soil, laboratory technique, medium and season as factors contributing to the phenomenon. The correlation data on moisture and χ^2 values show clearly that moisture content is not associated with χ^2 values. However, epidemics of high χ^2 values have been reported (9). The data presented in this study indicate that at certain samplings a larger number of high χ^2 values occur; particularly early in the summer. These could be accounted for, primarily, by the presence of pin-point colonies or excessive numbers of *Mucorales* on the sets. In fact, at all but one of the 41 platings of 36 samples in the experiments dealing with samples held one day, there is an excessive number of high χ^2 values. Further, the data for the summer of 1938 show that the causal factor does not affect the counts of actinomyces on the plates yielding the excessive numbers of high χ^2 values for counts of bacteria.

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HYDROIDS OF THE WESTERN CANADIAN ARCTIC REGION, 1935-1937[1]

BY C. MCLEAN FRASER[2]

The hydroids here reported upon were obtained by Sergeant H. A. Larsen of the Royal Canadian Mounted Police vessel *St. Roch* in July and August, 1936 and 1937*. While the vessel was stuck in the ice, Sergeant Larsen made a dredge which was lowered to the bottom and pulled along by the drift of the ice. The material was preserved and later deposited with the Pacific Biological Station, where it was sorted by Dr. Josephine F. L. Hart.

Although this material was not very extensive, it proved to be as interesting as any yet obtained from the Canadian Arctic. Of the five species in the collection, two appear to be new and one of these belongs to a genus quite different to anything previously described.

In Dease Strait, 68° 58′ N, 106° 20′ W, in 40 fathoms, the four species *Calycella syringa* (Linnaeus), *Thuiaria similis* (Clark), *Thuiaria tenera* (Sars), and a new species of *Bonneviella*, were obtained, and off Cape Bexley, Dolphin and Union Straits, 68° 59′ N, 115° 40′ W, in 9 fathoms, the new genus *Meganema* with the new species *M. claviformis*.

Description of Genus and Species

Genus *Bonneviella*

Bonneviella gracilis new species. Fig. 1, a, b, c.

Trophosome. Zooids growing singly from a slender stolon that winds about on colonies of *Thuiaria similis*. Pedicels slender, varying in length, the longest 2.5 mm., without annulations except the one at the base of the hydrotheca. Hydrotheca slender with length varying from 2 to 2.5 times the width, greatest

[1] *Manuscript received September 26, 1938.*

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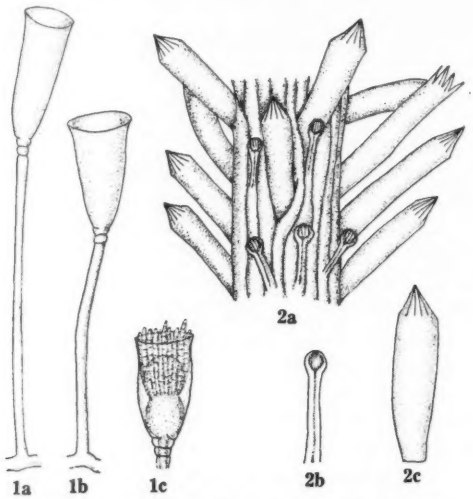
* *During the time that the Royal Canadian Mounted Police vessel "St. Roch" was on patrol duty in the Canadian Arctic from 1935 to 1937, Sergeant H. A. Larsen, in charge, took a number of hydrographic observations with instruments supplied by the Pacific Biological Station, and also made some collections of biological material. The data and collections have been deposited with the Station, where they are being examined in part, while certain groups are being submitted to specialists for study. A series of reports will be prepared for publication.*

To the Royal Canadian Mounted Police and to Sergeant Larsen, the Fisheries Research Board of Canada is greatly indebted for these collections, which will contribute to the biology and hydrography of Arctic waters.

W. A. Clemens, Director, Pacific Biological Station, Nanaimo, B.C.

length 1.0 mm., tapering very gradually from base to margin and sometimes slightly urceolate. Margin slightly flaring, entire. Hydranth with about 20 tentacles. These have rings of nematocysts almost like those on the tentacles of *Gonionemus*, although they are not so pronounced.



FIGS. 1 AND 2. *Magnification ×20.* FIG. 1. *Bonneviella gracilis.* **a** *and* **b,** *hydrotheca and pedicels.* **c,** *hydrotheca with hydranth.* FIG. 2. *Meganema claviformis.* **a,** *portion of fascicled stem to show hydrothecae and tentacular organs;* **b,** *a single tentacular organ,* **c,** *a single hydrotheca.*

NOTE: Apart from its generic characters this species bears little resemblance to other species of the genus. The relatively small size of the hydrotheca, which is campanulate rather than tubular, with an entire, even, flaring margin, sets it distinctly apart from all other species.

Gonosome. Not observed.

FAMILY CAMPANULINIDAE

Genus *Meganema* new genus

Trophosome. Colony fascicled, with sessile hydrothecae of the *Campanulina* type, arising from the central or axial tubes. These axial tubes are covered by more slender peripheral tubes from which extend long stalked nematophores or tentacular organs.

Gonosome. Unknown.

Meganema claviformis new species. Fig 2, a, b. c.

Trophosome. Colony stout, of much the same size throughout, up to 4 cm. in length, without branches, attached to sponges, barnacle shells, etc. The

peripheral tubes are about half the diameter of the axial tubes. Hydrothecae irregularly arranged, coming out from the axial tubes between the peripheral tubes; they are almost regularly tubular, varying more in length (maximum 1.25 mm.) than in width (0.3 mm.). There is no definite margin at the base of the opercular segments, which are ten in number, narrowing uniformly to a point from all sides as in *Campanulina*. The nematophores, or tentacular organs, are numerous, presenting a striking feature of the species; they come off the peripheral tubes, with long pedicels, 0.6 to 0.8 mm., less than 0.1 mm. in width. The terminal bulb is somewhat larger than, but not twice as large as, the diameter of the pedicel.

Gonosome. Not observed.

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The author wishes to express his thanks to Dr. W. A. Clemens and the Fisheries Research Board for the opportunity of examining the hydroids, and to Miss Ursula Dale for the drawings used in the illustration.

CUMACEA AND DECAPODA OF THE WESTERN CANADIAN ARCTIC REGION, 1936-1937[1]

By Josephine F. L. Hart[2]

Cumacea

Sixteen species of Cumacea were dredged at five localities southwest of Victoria Island, in the western Canadian Arctic region in 1936–37, by Sergeant H. A. Larsen, master of the R.C.M.P. schooner "St. Roch". Although the Canadian Arctic Expedition investigated part of the same area, only three species of Cumacea were obtained (1). Two of these were taken again and the third, *Diastylis rathkii* Kroyer, has subsequently (7) been divided into a number of subspecies and *D. oxyrhyncha*, of which young individuals occur in the present collection. The other species found seem to be new records for the area and are of interest as a link between the fauna of the North Atlantic and of Alaska, where rather extensive studies on the Cumacea have been made. On the whole, the species are North Atlantic or Arctic forms rather than Alaskan. The extension of range is therefore westward.

Details of distribution have been given by recent authors and thus only those localities are listed here that are of interest because of their proximity to the region concerned. The difficulty of positive identification in some instances has been increased by the predominance of immature forms in the collection.

Two species are of interest because of their comparative rarity: *Brachydiastylis nimia* Hansen would appear to have been taken only once before and then off East Greenland and *Cumella carinata* Hansen four times previously, the locality closest to the present one being at Ellesmere Land (79° 30′ N, 106° W). None of the other species is especially rare off Greenland or in Europe, and further investigation will probably obliterate the gaps between.

Family Leuconidae

Leucon nasicoides Lilljeborg

Locality. Dease Strait, 68° 58′ N, 106° 25′ W, 40 fms., July 25, 1937, 1 ♀ 4 mm.

Western limit. Gulf of St. Lawrence.

Leucon fulvus Sars

Locality. Dease Strait, 69° N, 106° 25′ W, 45 fms., August 7, 1936, 1 ♂ mature 5 mm., 4 ♀ 4 mm.

Dease Strait, 68° 58′ N, 106° 25′ W, 40 fms., July 25, 1937, 4 ♀ 4 mm.

Bernhard Harbour, 68° 50′ N, 114° 55′ W, 5 fms., Aug. 22, 1936, 1 ♂ mature 5 mm.

Western limit. Davis Strait, 66° 35′ N, 56° 38′ W.

[1] *Manuscript received September 26, 1938.*
Contribution from the Pacific Biological Station, Nanaimo, B.C.
[2] *Temporary Assistant, Pacific Biological Station, Nanaimo, B.C.*

Remarks. The male from Dease Strait has two teeth at the apex of the frontal lobe. Both males have three subequal lanceolate setae and one short seta on the third leg, agreeing with Hansen's (2) but not with Sars' (5) descriptions.

Leucon pallidus Sars

Locality. Dease Strait, 69° N, 106° 25′ W, 45 fms., Aug. 7, 1936, 1 ♀ 3.5 mm.

Dease Strait, 68° 58′ N, 106° 25′ W, 40 fms., July 25, 1937, 3 ♂ 4 mm.

Western limit. Davis Strait, 66° 35′ N, 56° 38′ W.

Leucon acutirostris Sars

Locality. Dease Strait, 69° N, 106° 25′ W, 45 fms., Aug. 7, 1936, 5 ♂ 4 mm., 1 ♀ 3 mm., 1 broken.

Dease Strait, 68° 58′ N, 106° 25′ W, 40 fms., July 25, 1937, 1 ♂ 4 mm.

Western limit. Davis Strait, 66° 35′ N, 56° 38′ W.

Leucon nasica (Kroyer)

Locality. Dease Strait, 69° N, 106° 25′ W, 45 fms., Aug. 7, 1936, 4 ♀ ovig. 8.5 mm., 20 ♂ and 116 immature.

Dease Strait, 68° 58′ N, 106° 25′ W, 40 fms., July 25, 1937, 1 ♀ 8 mm.

Bernhard Harbour, 68° 50′ N, 114° 55′ W, 5 fms., Aug. 22, 1936, parts of about 10.

Western limit. Beachy Island, Labrador, and southern Alaska.

FAMILY CAMPYLASPIDAE

Campylaspis rubicunda (Lilljeborg)

Locality. Dease Strait, 69° N, 106° 25′ W, 45 fms., Aug. 7, 1936, 4 ♀ 4.5 mm. and 3 mm., 3 ♂ 3 mm. and 4.5 mm.

Bernhard Harbour, 68° 50′ N, 114° 55′ W, 5 fms., 1 ♂ lacking abdomen.

Western limit. West Greenland.

Remarks. There are no definite teeth on the first and second pedigerous segments, and the propods are stouter than indicated by Sars' figures, but these are probably due to the immature state of the specimens, as the colour and the four spines on the dactylus of the second maxilliped are like *C. rubicunda*. Hansen (2) considers the latter character quite distinctive.

Campylaspis costata Sars

Locality. Dease Strait, 69° N, 106° 25′ W, 45 fms., Aug. 7, 1936, 1 ♂ 4.5 mm.

Dease Strait, 68° 58′ N, 106° 25′ W, 40 fms., July 25, 1937, 1 ♂ 3 mm.

Western limit. Irish Sea.

Remarks. The tubercles are more prominent than usual, the lateral carina being joined dorsally by a series of tubercles rather than by carina. Elongated

red-brown chromatophores outline the carina and tubercles, the margin of the carapace, the abdomen, and the appendages.

Family Nannastacidae

Cumella carinata Hansen

Locality. Dease Strait, 68° 58′ N, 106° 25′ W, 40 fms., July 25, 1937, 2 specimens 3 mm.

Western limit. Off Ellesmere Land, 79° 30′ N, 106° W.

Remarks. Both specimens are encrusted with a *Vorticella*-like ciliate.

Family Diastylidae

Diastylis spinulosa Heller

Locality. Dease Strait, 69° N, 106° 25′ W, 45 fms., Aug. 7, 1936, 1 ♀ ovig. 20 mm. uropods broken, 1 specimen 10 mm., 2 specimens 6 mm. (lack last pair of legs).

Dease Strait, 68° 58′ N, 106° 25′ W, 40 fms., July 25, 1937, 1 specimen 6 mm. Dolphin and Union Strait, 69° 5′ N, 115° 45′ W, 60 fms., Aug., 1937, 1 specimen 6 mm.

Western limit. Dolphin and Union Strait, 68° 50′ N, 115° W.

Remarks. All specimens have more and sharper spines than are described from European specimens. Calman (1) noted more numerous spines.

Diastylis goodsiri Bell

Locality. Dease Strait, 69° N, 106° 25′ W, 45 fms., Aug. 7, 1936, 2 specimens 8 mm. (no fifth legs), posterior part of slough of ♂, 13 mm.

Dease Strait, 68° 58′ N, 106° 25′ W, 40 fms., July 25, 1937, 1 specimen 5 mm. (no fifth legs).

Western limit. Dolphin and Union Strait, 68° 50′ N, 115° W.

Remarks. The small specimens have the carapace covered with fine spines and hairs.

Diastylis edwardsi Kroyer

Locality. Dease Strait, 69° N, 106° 25′ W, 45 fms., Aug. 7, 1936, 1 ♂ 6 mm., 2 specimens 4 mm. (no fifth legs).

Dease Strait, 68° 58′ N, 106° 25′ W, 40 fms., July 25, 1937, 1 ♂ 8 mm., 3 specimens 4.5 mm. and 3 mm. (no fifth legs).

Western limit. Baffin Land, 72° 38′ N, 77° 10′ W.

Remarks. One specimen has the point on the fifth pedigerous segment more pronounced than is usual.

Diastylis oxyrhyncha Zimmer

Locality. Dease Strait, 69° N, 106° 25′ W, 45 fms., Aug. 7, 1936, 1 abdomen of ♂ whose total length would be about 17 mm., 2 ♀ 13 mm., 5 specimens

10 mm., 3 specimens 8 mm., 12 specimens 5 mm. (no fifth legs) 23 specimens 3 mm.

Bernhard Harbour, 68° 50′ N, 114° 55′ W, 5 fms., Aug. 22, 1936, 2 damaged specimens, 5 and 3 mm.

Western limit. Baffin Bay, 72° 4′ N, 59° 50′ W.

Remarks. Although all the specimens are immature, the larger are so like Zimmer's (7) description and figure, that there seems to be little doubt as to their identity. The smaller specimens are definitely of the *D. rathkii* group and probably of the same species. Calman (1) records *D. rathkii* from Bathurst Inlet, 67° 35′ N, 108° 40′ W, and as *D. oxyrhyncha* was included then with this species, it is possible that both records are of the same species.

Leptostylis villosa Sars

Locality. Dease Strait, 69° N, 106° 25′ W, 45 fms., Aug. 7, 1936, 9 ♀ ovig. 4 mm., 100 ♀ 3.5–4 mm., 50 ♂ 4 mm.

Dease Strait, 68° 58′ N, 106° 25′ W, 40 fms., July 25, 1937, 7 ♂ 3.5 mm., 13 ♀ 3.5 mm.

Bernhard Harbour, 68° 50′ N, 114° 55′ W, 5 fms., Aug. 22, 1936, broken 1 ♂ and 7 ♀.

Western limit. Davis Strait, 66° 35′ N, 56° 38′ W.

Leptostylis ampullacea (Lilljeborg)

Locality. Dease Strait, 69° N, 106° 25′ W., 45 fms, Aug. 7, 1936, 1 ♂ 5 mm., 1 ♀ 4.5 mm., 1 specimen 4 mm.

Dease Strait, 68° 58′ N, 106° 25′ W, 40 fms., July 25, 1937, 1 ♀ 4.5 mm., 1 specimen 2 mm. (no fifth legs).

Western limit. Gulf of Maine.

Brachydiastylis resima (Kroyer)

Locality. Cape Krusenstern, Coronation Gulf, 10 fms., Aug. 1937, 1 ♀ 5 mm. (damaged).

Western limit. Baffin Bay, 72° 38′ N., 77° 10′ W.

Remarks. As pointed out by Hansen (2), there is a spinous process on the exterior margin of the second joint of the third pair of legs and the inner terminal seta of the exopod of the uropod is considerably longer than Sars' (5) figure.

Brachydiastylis nimia Hansen

Locality. Dease Strait, 69° N, 106° 25′ W, 45 fms., Aug. 7, 1936, 2 ♀ ovig. 3.5 mm., 4 ♀ 3–3.5 mm., 1 specimen 3 mm.

Western limit. East Greenland, north of Stewart Land, 70° 30′ N.

Remarks. One female is as described by Hansen (2), except that the teeth on the lateral margin of the carapace are somewhat more recurved than his figure would indicate. The other mature female and one of the immature,

has in addition, three square teeth, almost mushroom-shaped, between the large antero-lateral tooth and the base of the pseudorostral lobes. The remainder have four or five similar teeth on each side of the anterior part of the carapace.

Decapoda

The decapod collection from the vicinity of Victoria Island is a small one, comprising only seven species of shrimps. Six of these were obtained by the Canadian Arctic Expedition (4) from much the same area, and the seventh, *Argis dentata* Rathbun, has been taken in the Bering Sea (3) and off Baffin Land and in Hudson Bay (6).

Family Hippolytidae

Spirontocaris groenlandica (J. C. Fabricius)

Locality. Cape Bexley, Dolphin and Union Strait, 9 fms., ♂ 43 mm., ♀ 39 mm., rostrum broken.

Spirontocaris spina (Sowerby)

Locality. Cape Bexley, Dolphin and Union Strait, 9 fms., 2 ♀, 47 and 20 mm.

Spirontocaris phippsii (Kroyer)

Locality. Dease Strait, 68° 58′ N, 106° 20′ W, 40 fms., July 25, 1937, ♀ 37 mm.

Spirontocaris polaris (Sabine)

Locality. Cape Bexley, Dolphin and Union Strait, 9 fms., ♀ 35 mm. with isopod parasitic in right gill chamber.

Dease Strait, 68° 58′ N, 106° 20′ W, 40 fms., July 25, 1937, ♂ 53 mm. (no dorsal teeth on rostrum), ♀ 61 mm.

Spirontocaris fabricii (Kroyer)

Locality. Cape Bexley, Dolphin and Union Strait, 9 fms., ♂ 45 mm., ♀ 50 mm.

Cape Krusenstern Harbour, Coronation Gulf, 10 fms., Aug. 12, 1937, 5 ♂, 47, (2) 40, 36, and 24 mm. (rostrum broken).

Family Cragonidae

Argis dentata (Rathbun)

Locality. Cape Krusenstern Harbour, Coronation Gulf, 10 fms., Aug. 12, 1937, 12 mm.

Remarks. This immature specimen was kindly identified for the author by Dr. Belle A. Stevens, to whom grateful thanks are extended.

Sabinea septemcarinata Sabine

Locality. Cape Bexley, Dolphin and Union Strait, 9 fms., 1 damaged specimen about 25 mm.

Dease Strait, 69° N, 106° 25′ W, 45 fms., Aug. 7, 1936, 2 ♀ 18 mm.

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STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

VI. ON THE LETHAL EFFECTS OF SOME NITROGENOUS CHEMICALS ON THE FREE-LIVING STAGES OF SCLEROSTOMES[1]

By I. W. Parnell[2]

Abstract

The effect of ten chemicals, containing a nitrogen radicle, on the free-living stages of Sclerostomes is discussed. Chloropicrin is the most lethal chemical yet tested; under the conditions of these tests, one part of chloropicrin will sterilize approximately 2,300 times its weight of fresh faeces, but its disadvantages weigh against its practicability for farm use. Aniline will sterilize about 525 times its weight of fresh faeces when undiluted and 800 or 900 times its weight as a very weak solution. Calcium cyanide, whose value is also limited by its danger, will sterilize 530 times its weight of fresh faeces. Pyridine is slightly more effective when applied undiluted or as a strong or medium strength solution; it will then sterilize about 400 times its weight of fresh faeces. Ammonium carbonate will sterilize approximately 50 times its weight of fresh faeces. Ammonium chloride, cupric nitrate, and ammonium nitrate will sterilize approximately 30, 25, and 21 times their own weight of fresh faeces respectively. They are, however, more effective if applied as medium strength solutions, and cupric nitrate in lesser amounts causes the death of many larvae after they have reached the third stage, although the chemical was added to the fresh faeces. Ammonium sulphide, as a 15% solution, will sterilize nearly 14 times its weight of fresh faeces. Saponin probably has no lethal value against Sclerostomes chemically, although physically it may affect them.

This paper reports on the lethal values against Sclerostomes in fresh faeces of some chemicals containing a nitrogen radicle, *viz.*, chloropicrin, aniline, calcium cyanide, pyridine, ammonium carbonate, ammonium chloride, cupric nitrate, ammonium nitrate, ammonium sulphide, saponin.

Previous papers (19, 21) have reported on urine and ammonia and on ten of the more common nitrogenous artificial fertilizers.

Of the chemicals discussed in this paper, ammonium nitrate is the only one which is commonly used as a nitrogenous fertilizer, but it is most frequently used as a constituent of a mixed fertilizer. However, chloropicrin, calcium cyanide, and pyridine are all reported to act as plant stimulants when used in smaller quantities than those necessary to check growth.

The technique used to obtain the data reported in this paper was that previously described (18, 22), except that a few of the cultures made with the larger quantities of chloropicrin and calcium cyanide (Series CXVIII, CXXXIV, and CVL) were not kept in the constant temperature room, owing to its lack of ventilation.

Some of the chemicals discussed in this paper have been tested for their effect on plant nematodes, but practically no work has been done with them in regard to the bursate nematodes. However, some of the results on plant

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nematodes and other pests are sufficiently comparable to be worth considering; especially is this true of chemicals that might be tried on grassland, where they might serve two or more purposes at the same time. It must be remembered that a nitrogenous chemical may make the interpretation of results on plant nematodes difficult because of the stimulation of the host plant by nitrogen.

The use of chloropicrin in the control of plant nematodes has been chiefly against the species that attack pineapples and bulbs. In pineapple fields (6, 7, 9) *Heterodera radicicola* has been reduced by about 90% when chloropicrin has been used at the rate of 120 to 170 lb. per acre, especially when it has been applied in holes not more than 18 in. apart. When the land was covered with mulching paper its effectiveness was increased with the length of time the gas was retained. When the amount was increased to 350 lb., increased control resulted.

With holes 12 in. apart and no mulching paper, it has been shown (14) that 163 lb. per acre of chloropicrin was effective in controlling *H. marioni*; under similar conditions 1,000 lb. of calcium cyanamide was not effective. According to the author's technique, the ratio of effectiveness between chloropicrin and calcium cyanamide against Sclerostomes is almost 1 : 50 (20). *H. marioni* has been controlled (8) by 0.6 cc. and over of chloropicrin in covered pots holding 12 kg. of sandy soil. Chloropicrin has also the value of being lethal to wireworms (23), and although only slightly soluble in water, it is moderately effective when applied as a 1 : 3,000 solution, and more so when made into an emulsion with an equal quantity of fish oil and then mixed with water at 1 : 500 (24); in this form it has not yet been tried against Sclerostomes.

Calcium cyanide is frequently used against rodents and some of the larger insect pests. In pot trials against plant nematodes (13) it has been shown to be of value. However, against *H. schachtii* calcium cyanide was inferior to carbon disulphide (4) and drained creosote salts; carbon disulphide will be discussed in a subsequent paper, where it will be shown that against horse Sclerostomes the order of efficiency is reveresd. Edwards (5) also found that 336 lb. per acre of calcium cyanide was ineffective against *Anguillulina dipsaci*. It may increase the yield of potatoes (10), and used at the rate of 750 to 2,000 lb. per acre (11), it substantially reduced nematode infection; on lightish loamy soil, with the other conditions favourable, 900 to 1,200 lb. increased the number of plants free from eelworm galls to about 90%.

Sodium cyanide, also because of the hydrocyanic acid gas which it liberates, especially when mixed with ammonium sulphate (2, 25), has been recommended for sterilizing small areas of soil. More cyanide is neutralized by a heavy than by a light soil (17). In excessive quantities it may retard the germination of seeds, although in small quantities it may be a stimulant. Potassium cyanide, which also liberates hydrocyanic acid, has been reported (1) to control *H. marioni* when used at the rate of 800 lb. per acre.

Pyridine and aniline do not appear to have been tested as lethal agents in soil, but they have both been tested against fly larvae in manure (3). Used

at the rate of about 80 lb. of solution per 10 cu. ft. of manure, pyridine, as a 1 : 100 and as a 1 : 500 aqueous solution, was reasonably effective, but was not as a 1 : 1,500 solution. Aniline at the same rate was also successful when used as a 1 : 400 or stronger aqueous solution, and as a 1 : 500 solution it caused a death rate of about 80%.

Ammonium carbonate as a 1% solution has been reported (12) to cause the death of hookworm ova in five days. Ammonium carbonate, ammonium chloride and saponin as 2% solutions have been found ineffective (14) against *Anguillulina (= Tylenchus) dipsaci.*

Cupric nitrate with potassium cyanide at the rate of $\frac{1}{4}$ lb. of each in 10 gal. of water for 10 sq. ft. reduced the infection of *H. marioni* (14) and *Ditylenchus dipsaci* (16). Cupric nitrate as a 2% solution, but not as a weaker solution, was also lethal to *Tylenchus dipsaci* (15). Saponin as a 2% solution was not toxic to *T. dipsaci* (15).

Table I shows the values of the controls, which are indicated in Figs. 1 to 9 by the Roman numerals.

Chloropicrin

Chloropicrin, a tear gas, which is also known as trichloronitromethane and nitrochloroform, is the most lethal chemical that has yet been tested against Sclerostome eggs or larvae. It was added to fresh faeces in quantities of 0.001 to 25.0 cc. It has a specific gravity of 1.69.

Fig. 1 shows the effects on the numbers and condition of the third-stage Sclerostome larvae finally obtained after treating fresh faeces with chloropicrin. Although 0.005 cc. very considerably reduced the number of the larvae, several reached the infective stage and many survived, even when several cubic centimetres of chloropicrin were added to the culture. This irregularity makes it very difficult to know exactly what quantity of chloropicrin is necessary to sterilize fresh horse faeces against Sclerostomes, but it is probably about 0.01 cc. to 40 gm. of faeces, equivalent to 0.043% by weight of the faeces.

These irregular results are somewhat similar to the results obtained with other chemicals that produce a lethal gas, some of which will be discussed in the next paper of this series.

The unpleasant effects on man, and even possible danger, will limit the value of this chemical for controlling Sclerostomes.

Aniline

Fig. 2 illustrates the results obtained with aniline, which is also known as aminobenzene or phenylamine. Aniline was tested undiluted and in dilutions up to 1 : 1,000. It has a specific gravity of 1.022.

Undiluted, it was tested in quantities of 0.001 to 25.0 cc. Quantities of 0.005, 0.01, and 0.015 cc. each sterilized one culture, and 0.0075 cc. sterilized two cultures, but others treated with up to 0.033 cc. contained

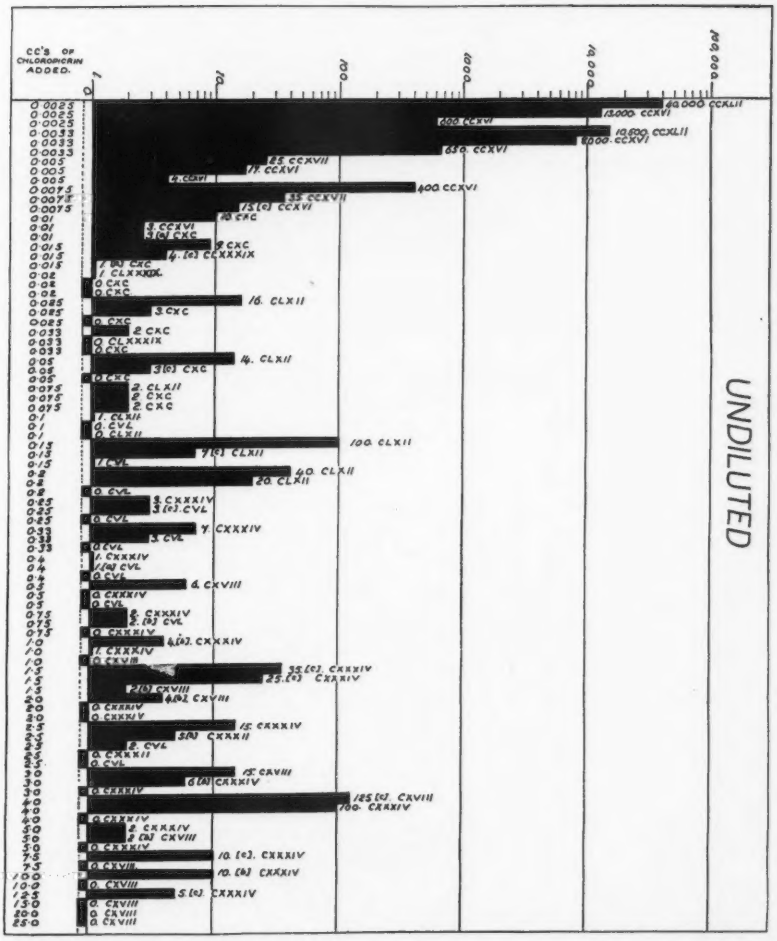

FIG. 1. *Results of tests with chloropicrin, undiluted, on cultures of fresh horse faeces. Roman numerals refer to the controls shown in Table I. a, after a number means that all, or practically all the larvae were dead; b, that a considerable proportion of the larvae were dead; c, that a few larvae were dead; d, that some Sclerostome larval debris, such as sheaths, was isolated, but not counted; e, that some live larvae were isolated, some of which were probably exsheathed Sclerostomes, and, therefore, not counted. The letter e is used only when inclusion of exsheathed larvae would have significantly increased the count of ensheathed larvae. The letters have the same significance in subsequent figures.*

numerous larvae, although very considerably less than in the controls. A few larvae survived in three cultures treated with 0.05 cc. With 0.075 cc. (equivalent to 0.19% of the weight of the fresh faeces) and over, the cultures were almost completely sterilized, although in some a few third-stage larvae were recovered.

TABLE I

CONTROLS FOR CULTURES TABULATED IN FIGS. 1 TO 9

Series No.	Date cultures made	Days kept in C.T room	Average number of larvae isolated	Series No.	Date cultures made	Days kept in C.T. room	Average number of larvae isolated
	1935				1937—*Conc.*		
XII	23 April	30	42,000	CXCI	8	48	28,000
XIV	21 May	23	29,000	CXCII	8	55	12,000
XV	21	36	18,000	CXCIV	12	51	31,000
XIX	31	32	13,000	CCVII	17 February	40	52,000
XX	31	33	12,000	CCVIII	18	49	55,000
XXII	5 June	39	25,000	CCX	23	44	35,000
XXVI	9 July	18	48,000	CCXVI	10 March	40	62,000
XXXIII	19	14	24,000	CCXVII	10	29	52,000
XXXV	22	84	23,000	CCXX	16	55	75,000
XXXVI	23	92	23,000	CCXXXVII	12 April	24	122,000
LII	22 November	25	62,000	CCXXXVIII	13	23	62,000
	1936			CCXXXIX	15	25	62,000
				CCXL	11 May	20	22,000
				CCXLII	18	27	35,000
LXXI	16 January	22	60,000	CCXLIII	18	27	91,000
LXXXII	18 February	25	16,500	CCLX	14 July	62	39,000
LXXXVIII	5 March	22	15,000	CCLXI	15	71	54,000
XCI	11	19	22,500	CCLXIV	16	70	16,500
CII	1 April	20	27,500	CCLXXVIII	12 October	23	28,000
CIV	7	23	21,500	CCLXXIX	13	22	44,000
CXVIII	12 May	21	2,000	CCLXXX	14	21	37,000
CXXII	22	17	4,400	CCLXXXI	15	31	44,000
CXXVII	9 June	16	11,000	CCLXXXII	18	28	45,000
CXXXII	12	22	8,500	CCLXXXIII	19	27	34,000
CXXXIV	17	37	40,000	CCC	22 November	36	55,000
CXXXVI	2 July	12	2,200	CCCI	22	36	35,000
CXXXVII	2	15	5,400	CCCII	23	35	24,500
CVL	3 August	81	22,500	CCCVI	2 December	36	63,000
CLIII	21 October	33	27,500				
CLIV	22	32	36,000		1938		
CLV	22	42	76,000				
CLXII	5 November	49	39,000	CCCXX	11 January	37	31,000
CLXXVIII	8 December	55	100,000	CCCXXI	12	36	31,000
CLXXX	9	57	69,000	CCCXXII	13	35	51,000
CLXXXIV	17	56	52,000	CCCXXIII	19	40	28,500
				CCCXCI	20	39	23,500
	1937			CCCXXXIII	18 March	61	24,000
				CCCXXXIV	23	75	19,500
CLXXXIX	6 January	50	28,000	CCCLII	9 June	21	7,800 c.
CXC	6	47	10,500	CCCLIX	12 July	20	37,000

A 1:50 aqueous solution was tested in quantities of 0.5 to 25.0 cc. From most of the cultures that were treated with up to and including 10.0 cc., a few third-stage larvae were recovered, but when 2.5 cc. or more was applied, the numbers of larvae were small (2.5 cc. of solution contains 0.049 cc. of aniline, or just over 0.12%). With the weaker solutions very similar results were obtained.

As a 1 : 100 solution, 4.0 cc. very considerably reduced the numbers of larvae recovered, but even with two or three times the quantity of fluid a few larvae survived (5.0 cc. is equivalent to slightly over 0.12%).

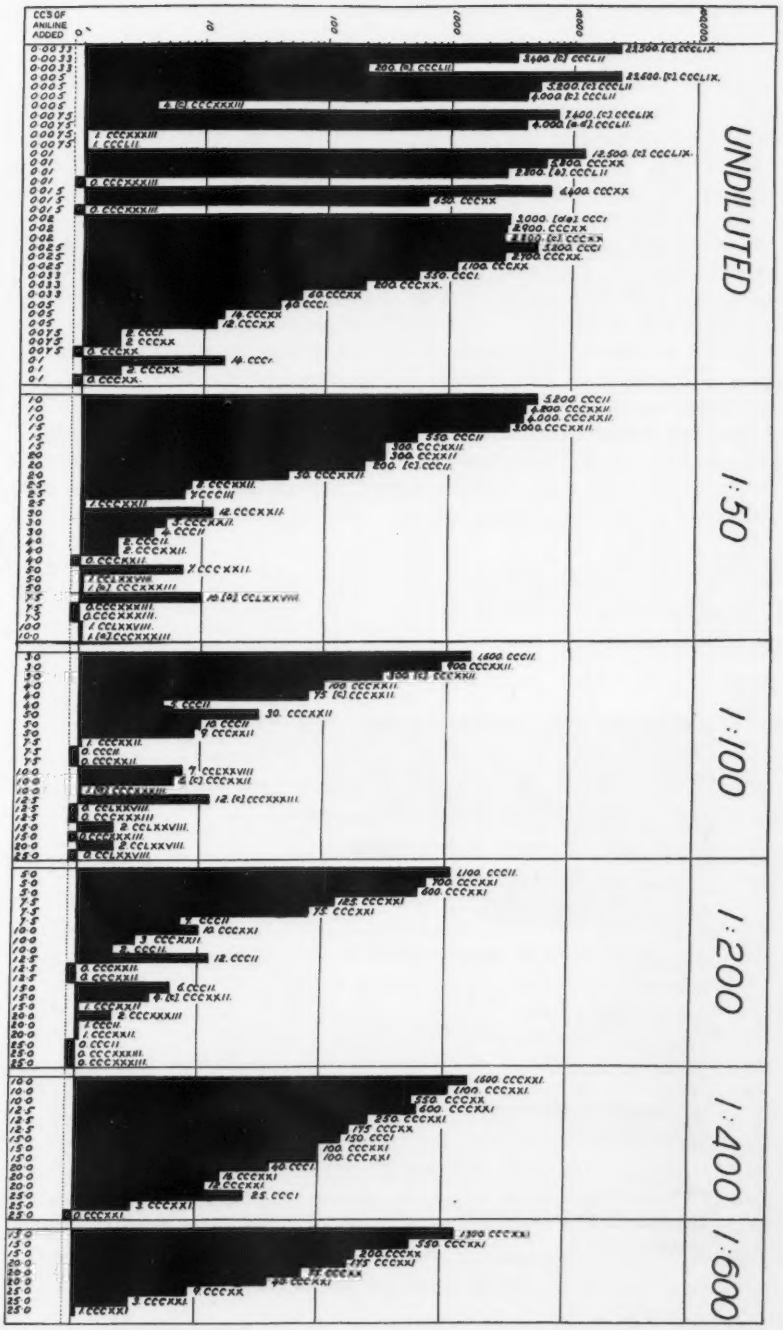

FIG. 2. *Results of tests with aniline, undiluted and in solution.*

The results obtained with a 1 : 200 solution suggest that 10.0 cc. (equivalent to 0.124%) is necessary to cause sterilization. When applied as a 1 : 400 solution, 15.0 cc. reduced the numbers of larvae to about a hundred, and 20.0 and 25.0 cc. almost sterilized the cultures (20.0 cc. is equivalent to almost 0.125%). Applied as a 1 : 600 solution, 25.0 cc. (equivalent to just over 0.1%) was effective, while 20.0 cc. and even 15.0 cc. greatly reduced the numbers.

The results obtained with a 1 : 300 and a 1 : 500 solution are not illustrated. When 15.0 cc. of a 1 : 300 solution and over was applied, only a negligible number of larvae was isolated; 15.0 cc. contains just under 0.05 cc. of aniline (equivalent to 0.125%).

As a 1 : 500 solution, the results confirmed the fact that about 0.125% of aniline, contained in 25.0 cc., is the quantity necessary to effect sterilization.

No cultures were sterilized when aniline was applied as a 1 : 800 or a 1 : 1,000 solution, but only small numbers survived the effects of 25 cc., and 20.0 cc. considerably reduced the numbers of larvae compared with the controls.

If these figures are averaged, they suggest that about 0.12% of the weight of fresh faeces is the amount of aniline necessary to cause sterilization.

Calcium Cyanide

Fig. 3 illustrates the sterilizing properties of calcium cyanide. It was tested in quantities of 0.0033 to 8.0 gm. One culture was sterilized by 0.02

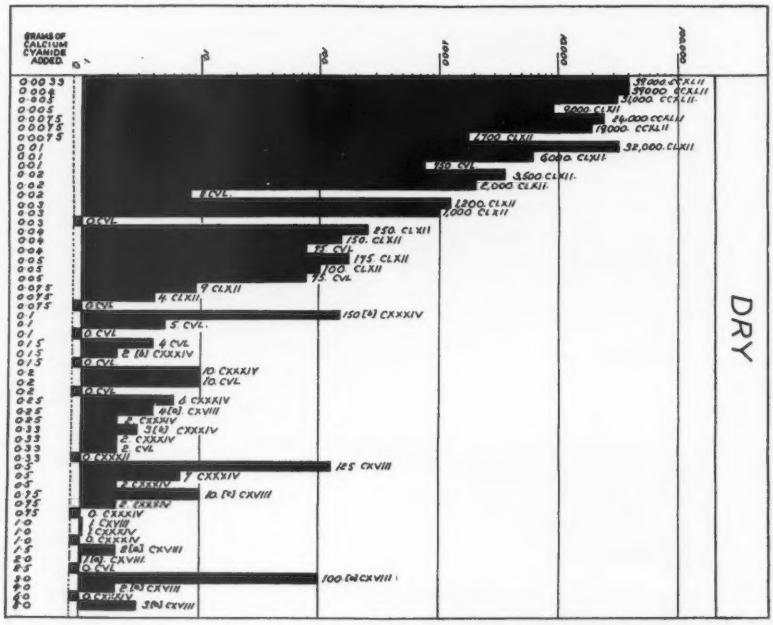

FIG. 3. *Results of tests with calcium cyanide, dry.*

and by 0.03 gm., and the numbers of larvae were very considerably reduced in all the cultures treated with 0.04 and 0.05 gm., but a very few larvae survived in some of the cultures treated with up to 1.0 gm. When larger quantities were used some reached the infective stage but subsequently died. On an average the results suggest that 0.075 gm. (equivalent to about 0.19%) is effective.

The danger to man and other mammals of the hydrocyanic gas given off by calcium cyanide will very considerably limit its use in practice.

Pyridine

Fig. 4 shows the results obtained with pyridine. For this series of cultures a pure form of pyridine was used, but if it were used in practice a cheaper grade would, of course, be essential.

Pyridine, undiluted, was tested in quantities of 0.02 to 25.0 cc.; it has a specific gravity of 0.99. The addition of 0.075 cc. very considerably reduced the number of larvae and 0.1 cc. (equivalent to 0.25%), practically sterilized them, although an occasional larva survived in cultures treated with up to 0.75 cc. Larvae did not survive in the cultures treated with 1.0 cc. and over.

When diluted, there was a considerable difference in the amount of pyridine required to kill the majority compared with that necessary to kill all the larvae. When the cultures were treated with a 1 : 2 aqueous solution, 0.25 cc. reduced the larvae very considerably and 0.33 cc. (equivalent to just over 0.27%) almost caused sterilization, but 1.0 cc. and over was required to make the cultures consistently free of larvae.

Applied as a 1 : 4 solution, 0.4 cc. considerably reduced the numbers of larvae; 0.5 cc. and over almost caused sterilization, while 2.5 cc. and over caused complete sterilization with the exception of one culture treated with 4.0 cc., from which 50 larvae, many dead, were recovered (0.5 cc. is equivalent to 0.25%).

As a 1 : 8 solution, 0.75 cc. was the smallest quantity of fluid that reduced the numbers of larvae very considerably; 1.0 cc. (equivalent to 0.28%) almost caused sterilization, but 75 larvae survived in one culture treated with 2.0 cc. From all except three of the cultures treated with 4.0 cc. and over, no larvae were recovered; from each of the three exceptions one larva was obtained.

Applied as a 1 : 20 solution, 1.5 cc. caused a very marked reduction, compared with both 1.0 cc. and the controls. The addition of 2.0 to 3.0 cc. almost sterilized the cultures, while larger quantities caused complete sterilization, except in two cultures from which three and one larvae respectively were recovered (2.0 cc. is equivalent to 0.24%).

The results obtained with a 1 : 50 solution are not illustrated. The addition of 4.0 cc. effected a marked reduction in the numbers of larvae, 5.0 cc. (equivalent to 0.24%) further reduced them to a negligible number, while none was recovered from any of the cultures treated with 7.5 cc. or over.

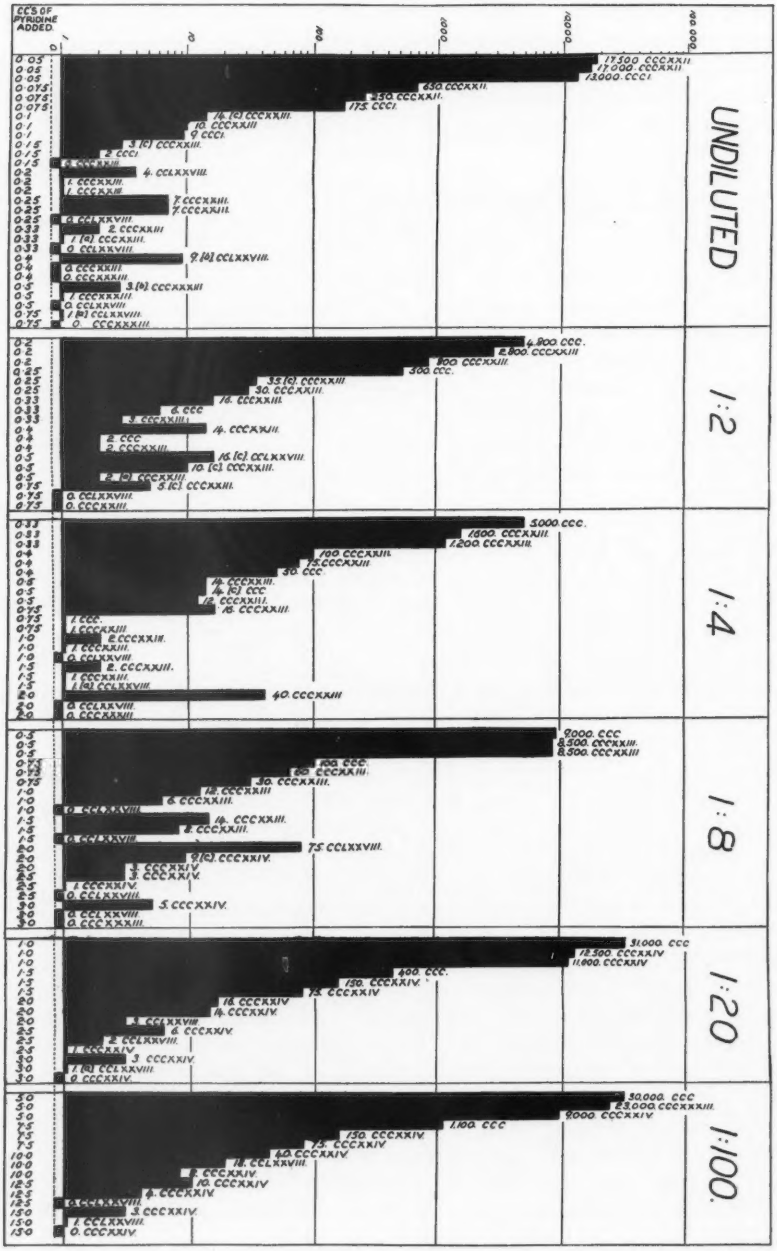

FIG. 4. *Results of tests with pyridine, undiluted and in solution.*

When added to the cultures as a 1 : 100 solution, 7.5 and 10.0 cc. reduced the numbers, the latter considerably, while 12.5 cc. and over practically sterilized the cultures (12.5 cc. contains about 0.33%).

Pyridine was also tested as a 1 : 200, a 1 : 300, a 1 : 400 and a 1 : 500 aqueous solution. The results are not illustrated.

Applied as a 1 : 200 solution, 15.0 and 20.0 cc. reduced the numbers considerably, while 25.0 cc. (equivalent to 0.31%) further reduced them to 50, 5, and 2. The weaker solutions were proportionately less effective.

An average of these results shows that about 0.27% of pyridine will sterilize fresh faeces under conditions similar to those existing for these cultures.

Ammonium Carbonate

The results obtained with ammonium carbonate are illustrated in Fig. 5.

With this chemical also, a few larvae escaped in cultures which had been treated with quantities considerably larger than that which caused death to most of the eggs and larvae.

Ammonium carbonate was applied dry in quantities of 0.2 to 8.0 gm. One culture was sterilized by 0.5, two by 0.75, and all by 1.0 gm., but in the cultures to which 1.5 gm. was added a few larvae survived. These results suggest that about 1 gm. (or 2.5%) of dry ammonium carbonate is necessary to cause sterilization.

Applied as a 1 : 2 solution, 2.0 cc. (equivalent to 1.9%) was effective. Again a few larvae reached the infective stage when larger quantities were added but most of these subsequently died.

When added to the cultures as a 1 : 4 solution, 4.0 cc. containing 0.89 gm. (equivalent to 2.2%) was effective; 2.5 and 3.0 cc. in one culture of each almost caused sterilization.

The results obtained with a 1 : 8 solution were somewhat irregular. Two cultures were sterilized by 5.0 cc., but 100 active larvae were isolated from the third. From the three cultures treated with 7.5 cc., respectively 500 dead, 200 active and 40, many of them dead, larvae were isolated. From one of the cultures treated with 12.5 cc., 175 dead larvae were obtained. An average of these results suggests that about 7.5 cc. (equivalent to 2.25%) will cause sterilization.

Applied as a 1 : 20 solution, the cultures treated with 15.0 cc. (equivalent to slightly over 1.8%) were almost sterilized, while 25.0 cc. was completely effective.

Added as a 1 : 50 solution, larger quantities considerably reduced the numbers of the larvae but did not cause sterilization.

The results suggest that on an average, ammonium carbonate must be used at the rate of about 2.1% of the weight of fresh faeces to produce sterilization against the free-living stages of Sclerostomes.

Ammonium Chloride

Fig. 6 illustrates the results obtained with ammonium chloride, which is also known as white salammoniac. It was tested dry in quantities of 0.2

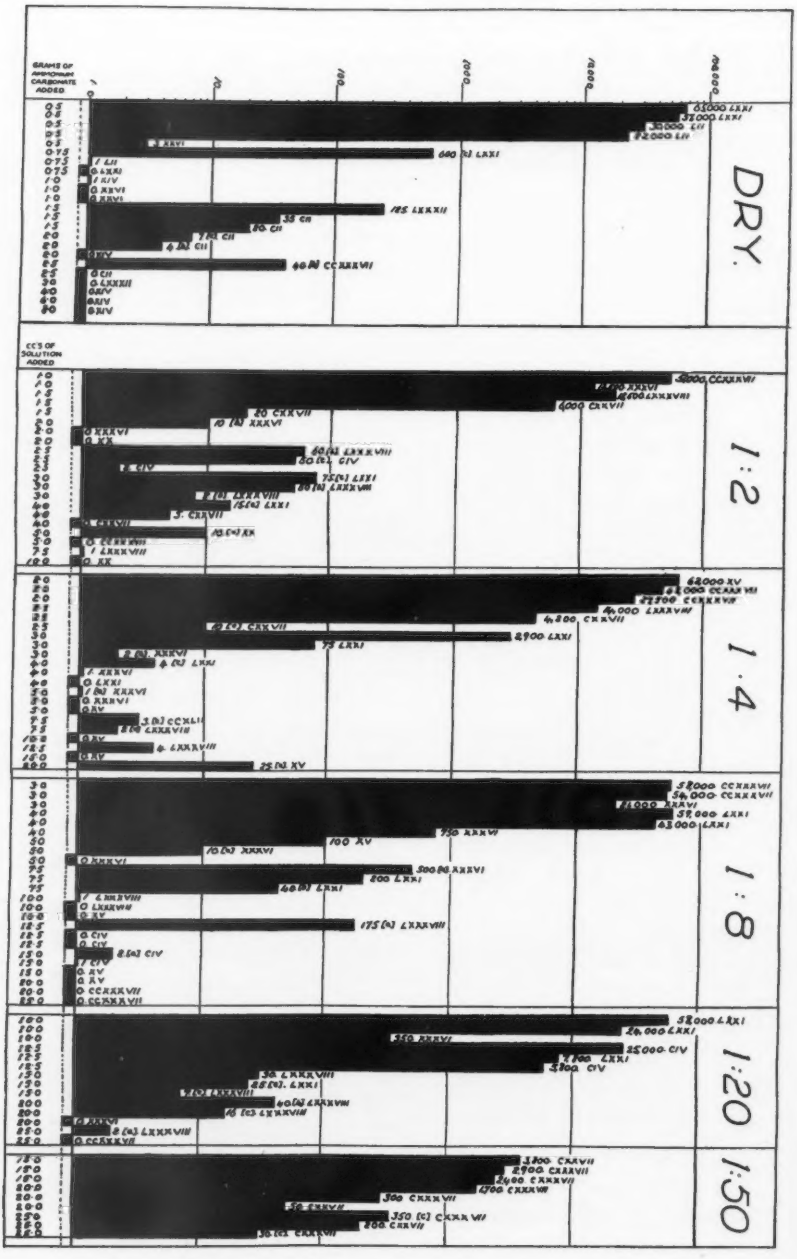

FIG. 5. *Results of tests with ammonium carbonate, dry and in solution.*

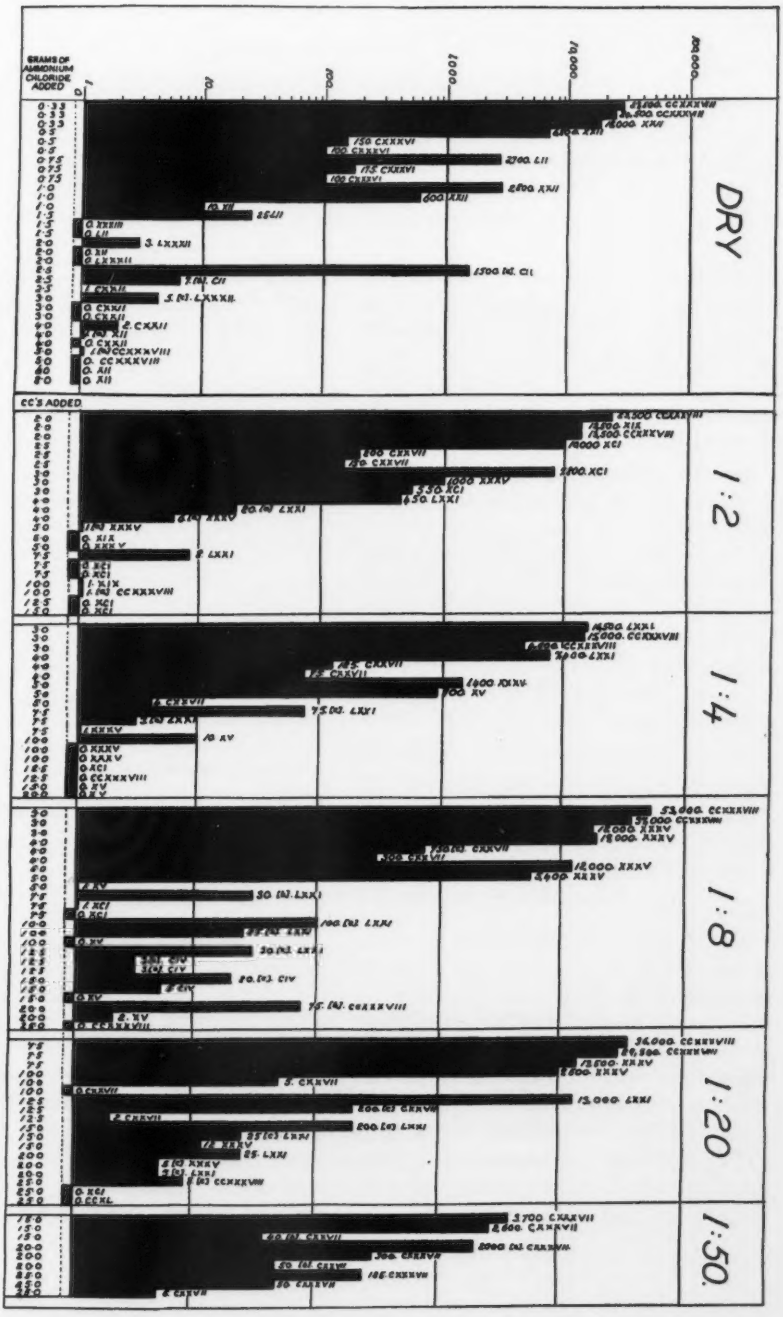

FIG. 6. *Results of tests with ammonium chloride, dry and in solution.*

to 8.0 gm. The results were irregular but it is probable that 1.5 gm. (equivalent to 3.75%) is approximately the amount necessary to sterilize fresh faeces. As one culture was almost sterilized by 1.0 gm. and all the cultures were almost sterilized by 1.5 and 2.0 gm., the culture that had been treated by 2.5 gm. and from which 1,500 larvae, a few dead, were recovered, need not be considered in making this estimate.

When added as a 1 : 2 solution, 5.0 cc. and over was effective and 4.0 cc. sterilized two cultures and reduced the number of larvae in the other (5.0 cc. contains about 4.7%).

When the cultures were treated with a 1 : 4 solution, 4.0 cc. considerably reduced the number of larvae in two cultures, and 5.0 cc. almost sterilized one culture. Two out of three cultures were sterilized by 7.5 cc., but 75 larvae, including a few dead, were isolated from the third. Averaging these results suggests that 7.5 cc., containing about 1.6 gm. (equivalent to 4.0%) is the amount of ammonium chloride, applied as a 1 : 4 solution, necessary to cause sterilization.

Although the results obtained with a 1 : 8 solution were more irregular, they suggest it is proportionately more effective than the stronger solutions. The addition of 4.0 cc. considerably reduced the numbers of larvae in two cultures, and 5.0 cc. sterilized one. In the series of cultures to which from 7.5 cc. (equivalent to 2.25%) to 20.0 cc. were added, some were sterilized, and from others a few larvae (mostly dead) were isolated.

Applied as a 1 : 20 solution, one culture was completely and one was nearly sterilized by 10.0 cc. Adding 12.5 cc. had a similar effect. From the cultures treated with 15.0 cc. and over, a few larvae were isolated, of which a few were dead (15.0 cc. is equivalent to slightly over 1.8%).

Added as a 1 : 50 solution, the larger quantities greatly reduced the numbers of larvae, but did not completely sterilize any cultures.

An average of these small-scale tests suggests that about 3.3% of ammonium chloride will sterilize fresh faeces against Sclerostomes, but that less is effective if it is applied as a medium strength solution.

Cupric Nitrate

Fig. 7 illustrates the effects of cupric nitrate ($Cu(NO_3)_2 . 6H_2O$) on the numbers and condition of the third stage ensheathed Sclerostome larvae from faeces that have been treated when fresh. Cupric nitrate, like many metallic sulphates and chlorides, may allow the larvae to reach the infective stage but subsequently may cause their death. This makes cupric nitrate difficult to evaluate, because it is impossible to know whether these larvae might have been capable of developing further if they had been swallowed by a suitable host, soon after they reached the third or infective stage.

Cupric nitrate was tested dry in quantities of 0.25 to 8.0 gm. One-half gram and less had little, if any, apparent effect on the vitality of the third-stage larvae, but 0.75 gm. caused many to die, and 1.0 gm. and over killed

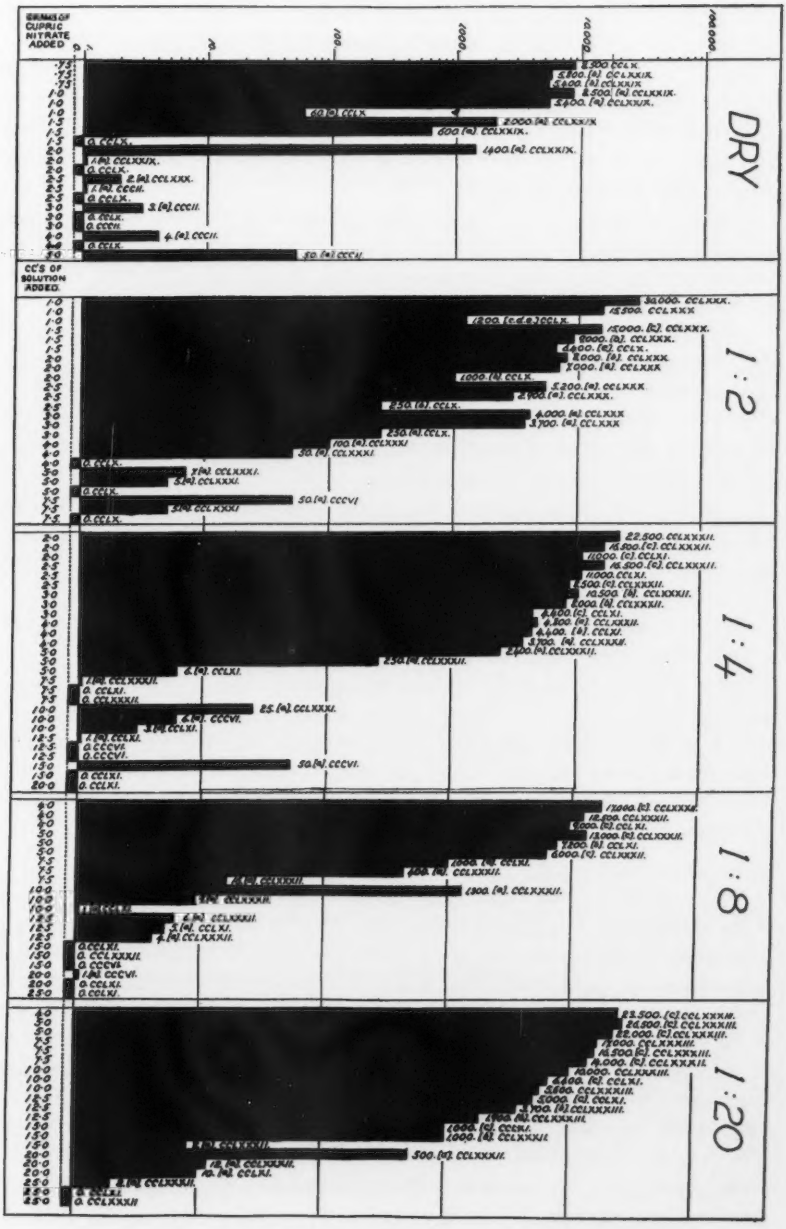

FIG. 7. *Results of tests with cupric nitrate, dry and in solution.*

them all. On an average, approximately 2.0 gm. (or 5.0%) of cupric nitrate was necessary to free from larvae the 40 gm. of fresh faeces.

When applied as a 1 : 2 solution, some of the larvae which reached the infective stage were killed by 1.5 cc., many were killed by 2.0 cc. and practically all by 2.5 cc. About double the latter amount made the cultures practically free of larvae, and 4.0 cc. greatly reduced the numbers (5.0 cc. is equivalent to about 5.25%).

Less than 2.0 cc. of a 1 : 4 solution appeared to have no effect on the vitality of the larvae which reached the infective stage; many died when 3.0 cc. was added and 4.0 cc. was lethal to the great majority. The cultures were practically free of larvae when 7.5 cc. (equivalent to 4.5%) was added.

When 4.0 and 5.0 cc. of a 1 : 8 solution were added, a few of the larvae which reached the third stage, subsequently died; when 7.5 cc. was added they all died. If the numbers of larvae isolated from the cultures treated with 7.5, 10.0 and 12.5 cc. are averaged, they suggest that 10.0 cc. (equivalent to 3.0%) is effective in making the cultures almost free from larvae when applied as a 1 : 8 solution.

Applied as a 1 : 20 solution, 20.0 cc. (equivalent to almost 2.5%) is probably effective in sterilizing the faeces. When 12.5 and 15.0 cc. were added, many larvae that reached the infective stage died, but when less was added only a few died.

The addition of the larger quantities of a 1 : 50 solution of cupric nitrate reduced the numbers of larvae, but did not sterilize the cultures.

An average of the results obtained with cupric nitrate suggests that to make the faeces almost free of third-stage larvae approximately 4.0% must be added to the fresh faeces, but that less is effective if it is applied as a medium strength solution, and that a half, or two-thirds of this quantity will cause the death of the larvae after they have developed to the infective stage, although treated in the egg stage.

Ammonium Nitrate

The results obtained with ammonium nitrate are illustrated in Fig. 8. This chemical was applied dry in quantities of 0.25 to 8.0 gm. If the effectively sterilized cultures made with 1.5, 2.0 and 3.0 gm. of ammonium nitrate are averaged with those which were made with the same quantities, but were not effectively sterilized, the results suggest that between 2.0 and 2.5 gm. (equivalent to about 6%) is the amount necessary to effect sterilization.

Applied as a 1 : 2 solution, 4.0 cc. sterilized one culture, 5.0 cc. sterilized two, and 7.5 cc. and over sterilized practically all the cultures (7.5 cc. is equivalent to about 7.0%). As some cultures were sterilized by 4.0 cc. and 5.0 cc. this estimate would probably be found to be too high.

Applied as a 1 : 4 solution, 7.5 cc. (containing about 4.1%) effectively sterilized the cultures, 5.0 cc. did not.

Added to the cultures as a 1 : 8 solution, the results are again irregular, but they suggest that 15.0 cc. (equivalent to 4.5%) is definitely, and 12.5 cc. may be, effective.

Applied as a 1 : 20 solution, 20.0 cc. very considerably reduced the numbers of the larvae in two cultures and sterilized one culture. The addition of

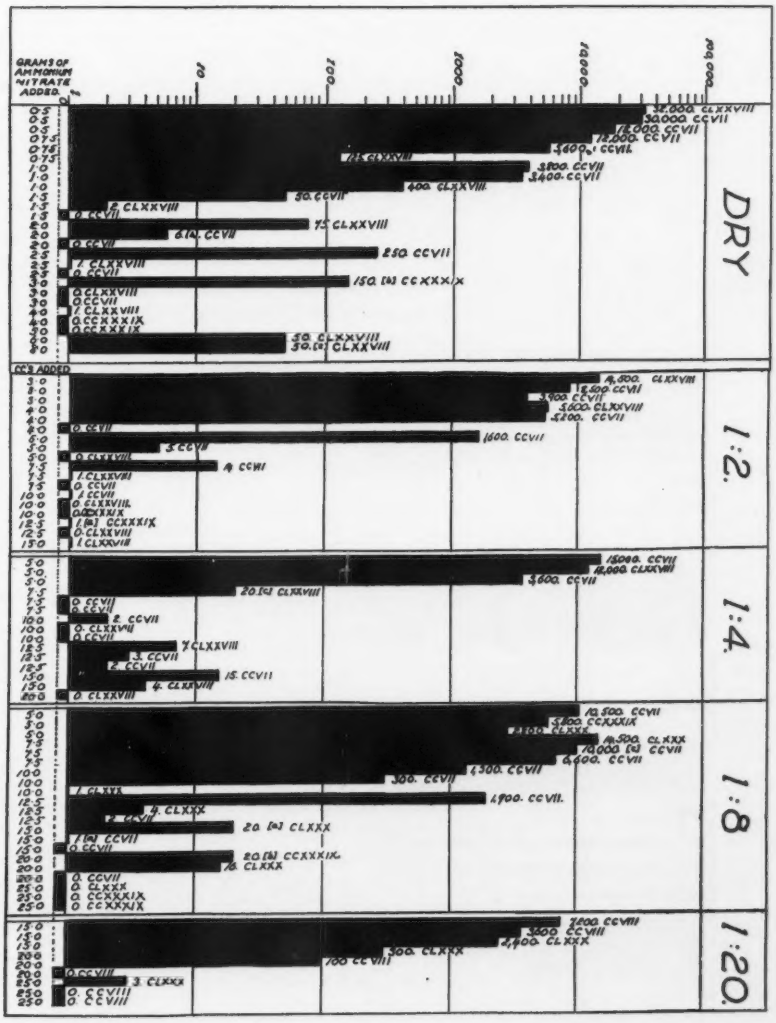

FIG. 8. *Results of tests with ammonium nitrate, dry and in solution.*

25.0 cc. (equivalent to slightly over 3.0%) of the same solution sterilized three cultures.

If the results are averaged, they suggest that approximately 4.75% of the ammonium nitrate is required to sterilize fresh faeces. They also show that ammonium nitrate should be used as a medium strength solution, when two-thirds of this amount may be effective.

Ammonium Sulphide

Ammonium sulphide was tested as "yellow" solution, containing 15% of ammonium sulphide in 85% of water. The results obtained with it are illustrated in Fig. 9. Without further dilution, 2.0 and 2.5 cc. each practically sterilized one culture, 3.0 cc. sterilized two cultures but 700 larvae were isolated from a third, while 4.0 cc. and over was effective.

When diluted with twice its volume of water, 7.5 cc. and over sterilized the cultures.

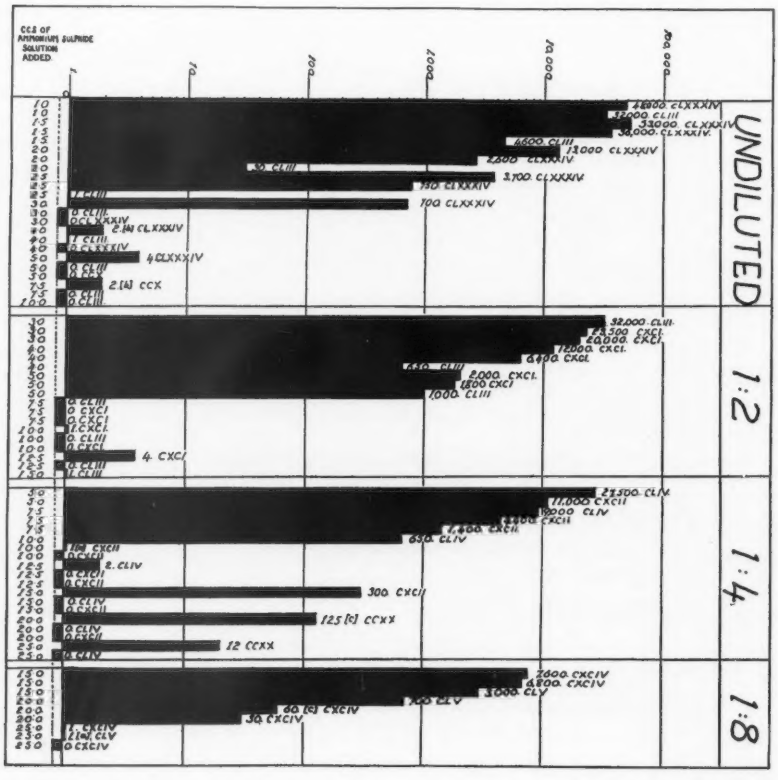

FIG. 9. *Results of tests with ammonium sulphide, as a 15% solution and diluted further.*

When four times its volume of water was added, two cultures were sterilized by 10.0, by 15.0 and by 20.0 cc., but three were sterilized by 12.5 cc.

When the dilution was increased to eight times the volume of water, 25.0 cc. caused sterilization, and 20.0 cc., reduction in the number of larvae.

Sterilization did not occur when the dilution with water was increased to 20 times.

The average of these results suggests that about 3.0 cc. of the yellow solution (containing 15% of ammonium sulphide) is effective in sterilizing 40.0 gm. of fresh faeces. It is probably slightly more effective when the solution is further diluted with up to eight times its volume of water.

Saponin

Saponin ($C_{27}H_{37}O_{16}N$) was tested dry and as 1 : 2, as 1 : 4, as 1 : 8, and as 1 : 20 aqueous "solutions". In some of the cultures made with saponin mixed in the faeces, both dry and in solution, the number of the larvae that were isolated was reduced, in a few cultures they were killed, and in a few they had exsheathed. As there was apparently little correlation between the quantity of saponin and the reduction in numbers or alterations of condition of the larvae, it is probable that saponin has little, if any, lethal action chemically, although its physical properties may affect the larvae. The possibility that saponin might add to the value of any lethal chemical that is difficult to bring in contact with the larvae, has not yet been tested.

The results with saponin were so irregular that they have not been illustrated.

Conclusions

Chloropicrin is the most lethal chemical that has yet been tested against Sclerostomes in fresh faeces, but its use, like that of calcium cyanide, which also is extremely lethal, will be limited by the difficulty of confining the gas and by its effects on mammals. If a sufficiently cheap grade of aniline or pyridine were available, their use might be practicable on many farms, especially if they could be used for fly control at the same time. The practicability of using ammonium carbonate, ammonium chloride, cupric nitrate, and ammonium sulphide to treat the top and sides of a midden, would depend largely on their cost, compared with that of comparable nitrogenous fertilizers. The price factor also applies to the practicability of using ammonium nitrate, which is slightly more lethal than potassium nitrate and sodium nitrate. Saponin probably has no chemical value as a lethal agent.

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